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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this research is to develop effective hemostatic devices for two difficult types of hemorrhage: (1) traumatic noncompressible (also known as truncal) hemorrhage; and (2) traumatic hemorrhage in the cold coagulopathic subject. The scope of this research will include both military and civilian trauma victims, particularly those suffering from hemorrhagic truncal injury and/or coagulopathic hemorrhage. During the third year (Y3) of this project, we continued to generate stocks of two clotting factors (fibrinogen and Factor XIII) needed for the development and manufacture of our hemostatic devices. In addition, we improved our prototypes to deliver foaming technology for the treatment of noncompressible hemorrhage. We performed efficacy testing of the dual (fibrin sealant-alginate) foam technology in our noncompressible model, and we currently are making modifications to both our model system and our foam technology to increase the resultant device utility. In Y4 we will continue to develop this foam technology for use in noncompressible hemorrhage. In addition, we will resume work on the treatments for cold coagulopathic hemorrhage with in vitro tensiometer studies, followed by in vivo studies in our cold coagulopathic porcine hemorrhage model.

15. SUBJECT TERMS
Trauma, hemorrhage, hemostasis, liver injury, biologics, clotting factors, biomaterial, bandage, wound, noncompressible, incompressible

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Note: any entry in this report with the label "Off protocol" at the beginning of the entry indicates that the entry represents work that was not directly associated with this DoD award, meaning that the work in that entry was not funded by this award.

1. INTRODUCTION

Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The subject of this research project is the treatment of hemorrhagic in two difficult clinical scenarios: (1) traumatic noncompressible (also known as truncal) hemorrhage; and (2) traumatic hemorrhage in the cold coagulopathic subject. The purpose of this research is to advance the technology of hemostasis, and to use preclinical large animal models to test hemostatic technologies on the two difficult types of hemorrhage named above. The scope of this research will include both military and civilian trauma victims, particularly those suffering from hemorrhagic truncal injury and/or coagulopathic hemorrhage, from both penetrating and blunt mechanism. In addition, the technologies under development in this research should be useful in nontrauma surgical procedures, both elective and emergency, in which solid organ, coagulopathic, and/or noncompressible hemorrhage might occur.

2. KEYWORDS

Provide a brief list of keywords (limit to 20 words).

Hemostasis
Swine
Trauma
Noncompressible
Hemorrhage
Coagulopathy
Recombinant
Clotting Factor
Fibrinogen
Hemodilution
Biomaterial
Polymer
Liver Injury
Exsanguination

3. OVERALL PROJECT SUMMARY

Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion. Key methodology used during the reporting period, including a description of any changes to originally proposed methods, shall be summarized. Data supporting research conclusions, in the form of figures and/or tables, shall be embedded in the text, appended, or referenced to appended manuscripts. Actual or anticipated problems or delays and actions or plans to resolve them shall be included. Additionally, any changes in approach and reasons for these changes shall be reported. Any change that is substantially different from the original approved SOW (e.g., new or modified tasks, objectives, experiments, etc.) require review by the Grants Officer's Representative and final approval by USAMRAA Grants Officer through an award modification prior to initiating any changes. [from the USAMRMC instructions: mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

Note: The description of the work performed during the past year will be organized according to the Tasks delineated in the project's Statement of Work (SoW), which has been reproduced in the Appendix A. For a timeline of Task execution, refer to the Gantt chart in Appendix B. For a list of acronyms used in this report, see Appendix D.

Task 1: Purification/generation of pd-FI and rFXIIIA2-a. [UNL]

During the last year of funding we have continued to purify multi-gram quantities of fibrinogen from human plasma for use in the foam studies. We use two different methods. Both start with a re-suspended cryoprecipitate of the plasma. The majority is further purified by EtOH precipitation of the solvent detergent treated cryo-supernatant. The resulting product has a protein profile and in vitro clotting properties typical of commercial preparations of fibrin sealant.

The second method utilizes ammonium sulfate to precipitate fibrinogen from the solvent detergent treated re-suspended cryoprecipitate. The resulting product contains what appears to be a one to one complex of fibrinogen and fibronectin. This observation was confirmed by size-exclusion chromatography. The complex exhibits enhanced clot strength at reduced concentrations and enhanced binding of exogenous cells. The result indicates it is possible to develop a more efficacious fibrin sealant for hemostasis and wound repair.

We also have continued to produce rFXIIIA2-a in quantities sufficient to continue research and development according to methods established in the Year 1 Annual Report. Four kilograms of recombinant FXIIIA2-a yeast wet cell paste were processed with a yield of 2-3 g of rFXIIIA2-a.

Task 2: Generation of ultrafine particles for tamponade carrier foam. [LNK]

See task 7; the focus of foam formulation has been on the use of alginate derivatives.

Task 3: Testing of candidate tamponade carrier & FS foams. [LNK & UNL]

Exploring formulations containing gelling agents instead of particles. During Y2 of this project second year, it was noted that the use of particles did not prevent a collapse of foam after 60 minutes. In order to keep the structural integrity of the foam, studies involving the addition of alginic acid sodium salt (alginate) to the foaming precursor solution and subsequently contacting the foam with a $CaCl_2$ solution to create a hardened gel structure were initiated during Y2 and continued during Y3. More detailed results are presented in Task 7 section.

Fibrin Sealant. Levels of pd-FI and rFXIIIA2-a continued to be carried out at to 21.9 mg/mL pd-FI and 0.72 mg/mL rFXIIIA2-a, from 9 mg/mL pd-FI and 0.36 mg/mL rFXIIIA2-a to investigate foam composition as part of task 3. The volume of fibrin sealant also was increased from 28 mL of LFS to 80 mL LFS to accommodate the foaming agent. The increased strength of the additional materials was demonstrated with TEG. *In vivo* efficacy testing of this new formulation will be performed during Y4. More detailed results are presented in Task 7 section.

Task 4: Testing of single foams in swine (tamponade carrier & FS foams separately). [UNMC]

In Y3Q1, four swine undergoing noncompressible injury per the approved animal protocol were treated with calcium solution and carrier gas only, no alginate foam (control group) in order to determine the effect of the carrier gas on blood loss in the noncompressible model [*1*], and to determine whether there was any significant surface injury to the intestines from the calcium solution. Two subjects survived the one hour observation period with 1.6 and 1.9 L blood loss, and two subjects expired from exsanguination with 2.4 and 3.3 L blood loss (see animal records 189-192 in Appendix C for details). There were minor surface effects of the calcium solution to the small intestine, but no evidence of necrosis. Insufflation of carrier gas appeared to have efficacy in 2 of 4 subjects, consistent with the recent report from another group of investigators, who observed that injection of a space-occupying nonresorbable urethane foam can improve survival in their porcine model of noncompressible hemorrhage [*2*].

In Y3Q2, testing of single-foam (alginate) formulations was performed in 12 swine; see detailed summaries of swine no. 193-204 in Appendix D. The *in vitro* and technical aspects of the foam treatment development is elaborated under Task 7 below. The heuristic foam testing progressed to the point at which a testable formulation was obtained for a formal trial of treatments in the noncompressible hemorrhage model.

In Y3Q3, single foam formulations from calcium alginate developed in Y3Q2 were combined with fibrin sealant foams during past quarter and underwent testing in the noncompressible hemorrhage model [*1*]; see task 6 below.

Task 5: Development & engineering of dual foam candidate devices. [LNK & UNL]

The current applicator nozzle developed in Y2 continues to successfully deliver optimized LFS circumferentially coated alginate foam both *in vitro* and *in vivo*.

Task 6: Testing of dual foam in swine. [UNMC]

In Y3Q1, two swine undergoing noncompressible injury [*1*] per the approved animal protocol were treated with FS foam using Barbasol foam as a carrier. There was no observable treatment effect. In fact, the presence of the Barbasol foam appeared to inhibit clotting (please refer to the animal record of Swine #188 in Appendix D for details). Based on this finding and previous results with Barbasol foam, the investigators of this project decided to abandon the use of Barbasol foam as an FS carrier for the development of a treatment for noncompressible hemorrhage. Alginate-based foams developed by LNK were chosen as an alternative. Swine testing of dual foam iterations continued in the following quarters.

In Y3Q3, Testing of a dual foam formulation (calcium alginate foam + fibrin sealant foam) was performed using the noncompressible hemorrhage model in 7 subjects (see Appendix D, Summaries 211-217). Five swine (~70%) died during the post-injury observation period; 6/7 swine (~85%) lost >3 L blood. These results actually were worse than our no-treatment control group, suggesting that: (a) our treatment was having a negative effect on noncompressible hemorrhage; (b) something was wrong with our model; (c) a combination of both *a* and *b*; or (d) something else.

We had a suspicion that the existing rapid, high-volume crystalloid resuscitation protocol was detrimental to subject survival after injury, i.e., that we had a problem with the model. Based on recent developments on the utility of damage control resuscitation (DCR) in reducing combat-injury mortality [*3*], and the fact that the current TCCC Guidelines [*4*] recommend hypotensive resuscitation for serious battlefield injuries, we decided to test the effect of hypotensive resuscitation in our porcine noncompressible model. Eight subjects underwent off-protocol testing (see Summaries 218-220, 222, 223, and 226-228 in Appendix D) in which our standard hepatic portovenous injury was created, and then crystalloid resuscitation was administered at a much slower rate (~20 ml/min, compared to the protocol value of 150 mL/min). Interestingly, 6/8 subjects injured subjects (with no treatment other than IVF) survived the 3 h observation period, with an average blood loss of 1,425 ± 325 mL, much less than the typical blood loss of 3-4 L in subjects resuscitated with 150 mL/min.

This finding suggested that rapid administration of large volumes of crystalloid was detrimental to survival during hemorrhagic shock, which was consistent with published preclinical and clinical data. Beginning in Y3Q4, we have been performing some off-protocol work on our noncompressible hemorrhage model in order to obtain an injury mechanism that can produce ~50% mortality during the first 3 h after injury while utilizing a hypotensive resuscitation protocol. The preliminary results of this testing were submitted as an abstract to the 2015 meeting of the Central Surgical Association (see Appendix S), and a more complete report should be available for the next quarter report on this project. We will submit the appropriate paperwork to ACURO to initiate approval for any proposed changes to our animal protocol, as needed.

Task 7: Engineering of firm foams from alginate and alginate derivatives. [LNK]

In Y3Q1, one of the observations made about the alginate foam used previously in the swine surgeries was that the final volume of the foam at the end of 1 hour survival studies was low (about 500 mL) and needed to be increased to several liters in order to provide better hemostatic effects.

The sodium alginate product previously used was purchased from Sigma Life Science (Alginic acid sodium salt, from brown algae – A1112). This alginate was used in the following formulation for producing the expanding foam: 6.5% sodium alginate, 0.2% xanthan gum, 0.9% tween 20 in DI water. The foaming solution was combined with 20 grams of liquid butane as a propellant to produce 5 liters of expanding foam in atmospheric pressure and room temperature. After much experimentation it was decided that alginate from Sigma Life Science was limited in producing firmer foam under conditions used in the swine survival studies.

Alginates are naturally occurring polysaccharides that are derived primarily from seaweed. They are composed of linked β-D- mannuronic acid (M blocks) and α-L-guluronic acid (G blocks) monomers along the polymer backbone. The ratio of G to M blocks in alginate product is dependent on the source from which the alginate was produced (i.e. type of seaweed and different parts of the plant). Both G and M block have carboxylic groups that form salts such as sodium alginate. Sodium alginate is soluble in water and forms a viscous liquid. The viscosity of sodium alginate solution is dependent on alginate concentration and the length of the polymer chains. Gelling process occurs when divalent metals such as calcium displace sodium and form a link between G blocks in adjacent polymer chains. Consequently, alginate products with higher G blocks percentage produce much firmer gels.

Two new alginate candidates one pharmaceutical grade and the other food grade with higher G block content produced by FMC BioPolymer (Philadelphia, PA) were chosen as a replacement. Comparison between the two showed that the initial solution viscosity for the food grade product was much higher than that for the pharmaceutical grade product and hindered experimentation and dispensing from the foam dispenser. Also, the FMC pharmaceutical grade alginate produced sufficiently firm foam and as a result was chosen as the new source for Alginate in the foam formulation.

The results of some of swine surgeries suggested toxicity due to one of the components in the foaming process (i.e. foam formulation components or calcium chloride). A series of experiments were performed to investigate the source of the toxicity. It was suggested that the toxicity was produced by excess calcium present after the gelling process was completed. A series of experiments were performed using the original foam formulation with Sigma alginate mentioned above to establish minimum amount of calcium needed for formation of stable rigid foam.

From previous experiment performed with the original foam formulation with Sigma alginate (6.5% sodium alginate, 0.2% xanthan gum, 0.9% tween 20 in DI water) it was established that for 300 mL of foaming formulation to produce stable rigid foam, a minimum of 180 mL of 0.6 molar calcium chloride solution (0.108 moles) was needed (reduction from 0.360 moles contained in 180 mL of 2 M solution used in previous swine surgeries). This amount of calcium chloride even in free form when applied in the swine abdomen did not produce any toxicity. This same amount produced much more rigid foam when applied to 3.85% FMC pharmaceutical grade alginate in the new formulation.

Experiments also were performed inY3Q1 to simplify the formulation further. Based on the

results of the experiments, it was discovered that the presence of tween 20 was integral to the foaming process and without it a non-cohesive grainy gel with very small volume was produced. In previous formulation of the foam with Sigma alginate, xanthan gum was used as a homogenizer. The experiments showed that the elimination of xanthan gum from the formulation with the new alginate did not adversely affect the foaming and gelling process. Consequently xanthan gum was eliminated from formulation. The final composition of the new foaming solution is as it follows: 3.85% sodium alginate, 0.6% tween 20 in DI water.

Experimental Setup for measuring foam rigidity. Established bench marks for the foam required a final volume of about 2 liters at 5 mmHg in the abdomen of the swine at the end of 1 hour survival study. To examine the rigidity of the foam in a laboratory setting, the following experimental setup was used in Y3Q1:

The expanding foam was dispensed into a large beaker of sufficient volume and immediately covered with a plate slightly smaller than the inner diameter of the beaker. Based on the surface area of the disk, sufficient enough weight was placed on the disk to produce a downward pressure of 5 mmHg. The beaker was heated prior and during the experiment using a space heater to simulate the abdominal temperature. The temperature was recorded inside the beaker by attaching a thermometer to the inner wall of the beaker.

Pressure calculations:

plate radius: 2.875 in   plate surface area: 25.967 in^2 bottle weight: 1140 g = 2.51 lb
1 mmHg = 0.01934 psi   2.51 lb/25.967 in^2 = 0.0967 lb/in^2 = 5 mmHg

To produce the expanding foam, the foaming formulation was dispensed from a pressurized cream dispenser with a modified nozzle containing about 20 g of butane as a propellant. Calcium chloride solution was dispensed through a needle inserted into the stem of the nozzle while the foam was being dispensed allowing for the complete mixing of dispensing foam and calcium chloride inside the nozzle prior to foam discharge. The tubing diameter used for the nozzle was chosen as such to allow placement inside the integrated nozzle used for dispensing procoagulant ingredients. Calcium chloride solution was pumped at the appropriate rate using a syringe pump equipped with three or four 60 mL syringes. The syringes were connected to a single delivery line through usage of interconnected valves.

1.) 300 mL of foaming solution was prepared as follows: 231 mL (231g) of 5% FMC pharmaceutical grade Algin in DI water, 30 ml (31.2 g) of 6% tween 20 in DI water, and 39 mL of DI water was mixed to produce the foaming formulation. This formulation was combined with 20 g of butane in a foam dispenser and was discharged simultaneously with 180 mL of 0.6 M calcium chloride at a flow rate of 102 mL per minute. About 4.0 L of gelled foam was produced in a 5 L beaker. A plate on top of the foam weighed down by a bottle with the proper weight was used to simulate pressure of 5 mmHg. After 1 hour the foam was compressed to 1400 mL. The experiment was performed at 35°C by placing a space heater directed at the 5 L beaker prior to start of the experiment and throughout the experiment. The final foam was elastic to the touch. The final volume of 1400 was much more than the 500 mL observed previously in laboratory setup and in the swine abdomen.
2.) An experiment was performed following the same formulation and set up as above with a difference of using 180 mL of 0.8 M calcium chloride in place of the 0.6 M solution to see if

the increased amount of calcium chloride improves the stiffness of the foam. After compressing the foam for 1 hour, 1400 mL of compressed foam was produced. It was concluded that the increased amount of calcium chloride did not add to the rigidity of the foam.

3.) Additional experiments were performed following the same formulation and setup as above but using increasing amounts of foaming solution with the addition calcium chloride solution and 21 g of Butane in order to achieve greater residual foam volume after compression for about 1h.

So in Y3Q1, we found that discharging 450 mL of foaming solution along with about 300 mL of calcium chloride solution and compressing the foam as described above for over 1 hour, the expanded foam reached its final volume of ~1900 mL at about 20 minutes and maintained at that level to the end of the hour.

In Y3Q2, improvement in alginate foam delivery was accomplished. As shown in in Y3Q1, utilizing high-G content pharmaceutical grade sodium alginate (Protanal LF200 FTS) produced by FMC Biopolymers (Philadelphia, PA) in the foam formulation resulted in a stable, rigid and expanding foam after experimentation with the percent sodium alginate in the formulation. The final composition of the new foaming formulation is as follows: 3.8% sodium alginate and 0.6% Tween 20 in DI water.

Using this formulation, effort was made in Y3Q2 to produce larger quantities of foam. Benchmarks requested by the surgical team for the foam aimed at a final compressed volume of 2-3 L at 5 mm Hg and 37 ˚C, after 1 h. The rigidity and final volume of the foam was measured by dispensing the foam into a large beaker, and immediately covered with a plate slightly smaller than the inner diameter of the beaker. Based on the surface area of the disk, enough weight was placed on the disk to produce a downward pressure of 5 mm Hg. The beaker was heated prior to, and during the experiment using a space heater to simulate the swine abdominal temperature.

Despite our *in vitro* compression results producing a final volumes of foam appropriate for the application in swine surgeries (V=3 L after compression), the final volume recovered from swine abdomen was generally limited to approximately half of the *in vitro* observed volumes (Swine #197 & #198 in Appendix D). Consequently, a setup capable of producing larger volumes of foam was needed.

In order to produce the larger amount of foam in Y3Q2, a larger 1 L cream dispenser was filled with 800 mL of foaming formulation with the following composition: 608 mL (608 g) of 5% FMC pharmaceutical grade sodium alginate in DI water (Protanal LF200 FTS) (final composition of 3.8% sodium alginate), 80 mL (83.2 g) of 6% tween 20 in DI water, and 112 mL of DI water. After many experiments, the optimal amount of butane propellant for this target foam load was found to be 120 g. When tested in the laboratory setup, this combination of foam and propellant produces a minimum of 3.5 L of compressed rigid foam at 37˚C and 5 mm Hg. As before, the calcium chloride solution was dispensed through a needle inserted into the stem of the nozzle while the foam was being dispensed, allowing for the complete mixing of dispensing foam and calcium chloride inside the nozzle, and prior to foam discharge. Considering the larger volume of formulation, the concentration of calcium chloride solution was increased to 1.14 M. It was delivered to the dispensing nozzle using a syringe pump equipped with four 60 mL syringes interconnected with valves to produce a single stream. The flow rate was set at 84 mL/min (21 ml/min per syringe × 4).

The larger 1 L dispenser with 800 mL of foaming formulation produced slightly more than 2

L of compressed foam with optimal rigidity when recovered from swine abdomen (Swine #199, Appendix D). To achieve a final foam volume close to 3-4 L target, two 1 L dispensers with 800 mL of foaming formulation each were used in sequence for swine surgery #200 (Appendix D). However, it was observed that due to the finite available volume in the swine abdomen, recovery of foam volumes larger than 2.5 liters was not possible anyway. Dispensing a larger amount of foam simply increased the intraabdominal pressure and increased foam rigidity. It is important to note that the majority of intraabdominal pressure observed was due to foam mass and not the propellant (vented through a tube inserted in the abdomen while dispensing the foam).

It was postulated in Y3Q2 that a lower flow rate coupled with a multiple dispensing point in the abdominal cavity was going to improve the effectiveness of the foam. Consequently, a trifurcated dispensing nozzle was devised to test this hypothesis. The trifurcated dispensing nozzle was designed in a manner to allow for equal flow rates from each exit point and absence of any internal bottle neck or choke points. Calcium chloride solution was delivered to this nozzle via three separate lines carrying the solution and dispensing it through three needles inserted into nozzle right after branching in the nozzle. Each calcium line was connected to a 60 mL syringe driven by a syringe pump at a rate of 27 mL/min. this allows for equal amount of calcium chloride to be available for the foam in comparison to delivery with a single nozzle. When using the trifurcated nozzle with a dispenser loaded with 800 mL of foaming formulation, a much reduced flow rate of foam was observed. The consistency of the gelled foam was also changed to result in a less cohesive and more noodle like foam.

Using CO_2 and NO_2 as a propellant for dispensing foam. It was suggested that a gas propellant such as CO_2 could be used as a replacement for butane as a propellant. The dispensers used for the foam were originally designed to be charged with pure nitrous oxide for dispensing whipped cream. As the charging valve was made for 8 g food grade NO_2 cartridges, an experiment was performed in Y3Q2 to examine suitability of NO_2 as a propellant for dispensing the foaming formulation. A 0.5 L foam dispenser was filled with 450 mL of foaming formulation with the following composition: 342 mL (342 g) of 5% FMC pharmaceutical grade sodium alginate in DI water, 45 mL (46.8 g) of 6% tween 20 in DI water, and 63 mL of DI water. The dispenser was charged with one 8 g NO_2 cartridge per manufacturer's recommendation. The dispenser was allowed to equilibrate for 3.5 hours as to provide sufficient amount of time for the gas to equilibrate with the liquid formulation. For the last 30 minutes the dispenser was suspended upside down to allow for the formulation to move toward the nozzle. The dispenser was discharged over a large beaker where the formulation dispensed rapidly out of the nozzle. However, unlike formulation dispensed with butane, no expansion of the formulation was observed and no foam was formed. No calcium chloride solution was dispensed along with the foaming formulation as the discharge was meant to explore the dispensing of foaming formulation and formation of expanding foam with NO_2 as propellant. It was concluded that despite achieving good discharge rates, this gas was not suitable for producing expanding foam.

Similar experimental setup was used in Y3Q2 to examine the feasibility of using CO_2 as a propellant for dispensing the foam. Again, unlike the formulation dispensed with butane, no expansion of the formulation and formation of foam occurred. Consequently, it was concluded that CO_2 also is not a suitable candidate for usage as a propellant in an expanding foam system. We hypothesize that at the high storage pressures and prior to delivery, the Tween/butane/formulation blend may form an emulsion, which is particularly amenable for foam formation due to rapid butane bubble formation on depressurization and discharge.

In Y3Q3, a foam formulation with the following composition was used in the swine

surgeries: a one-liter cream dispenser was filled with 800 mL of foaming formulation, with the following composition: 608 mL (608 g) of 5% FMC pharmaceutical grade sodium alginate in DI water (Protanal LF200 FTS) (final composition of 3.8% sodium alginate), 80 mL (83.2 g) of 6% Tween 20 in DI water, and 112 mL of DI water. The dispenser is filled with 120 g of butane as propellant. As before, calcium chloride solution was dispensed through a needle inserted into the stem of the nozzle, while the foam was being dispensed allowing for the complete mixing of dispensing foam and calcium chloride inside the nozzle prior to foam discharge. Considering the larger amount of formulation, the concentration of calcium chloride solution was increased to 1.14 M. It was delivered to the dispensing nozzle using a syringe pump equipped with four 60 mL syringes interconnected with valves to produce a single stream. The flow rate was set at 84 mL/min (21 ml/min per syringe x 4).

One of the observations made during the swine surgeries was the presence of excess propellant after the foam in the dispenser was delivered into the swine abdomen. The excess gas rushing into the abdomen quickly increased the inter-abdominal pressure to about 40 mmHg (typical inter-abdominal pressure is about 5 mmHg). It was also observed that a longer duration of foam delivery produced a more pellet-like consistency (most likely due to a drop in temperature in the dispenser, and a concomitant increase in the viscosity of the formulation). The pro-coagulant delivered along with the foam is typically dispensed in about 45 seconds and as such, dispensing the entire large canister cannot be significantly longer than this duration.

To remedy the issues above, a simple solution was devised during Y3Q3. The propellant amount was reduced from 120 g to 85 g to reduce the amount of excess propellant after dispensing the entire foam formulation content in the canister. To compensate for removed propellant and improve discharge duration and the consistency of foam produced, the dispenser was also warmed to approximately 30°C from room temperature prior to dispensing. Experimenting with this new set up showed a significant improvement in foam delivery time, and still produced a cohesive and intertwined foam mass into the swine abdomen.

During Y3Q3, LNK continued to support the surgeries by having at least one member present during the procedures to offer logistical support as needed with the foam device operation, and also carried out the required thromboelastography (TEG) tests to characterize the clotting power of the subjects at different stages of the procedure.

Task 8: Testing of dual foam in swine noncompressible model (laparotomy with 2˚ closure). [UNMC]

See discussion under Task 6.

Task 9: Delivery of candidate field-ready dual foam device. [LNK & UNL]

This task has yet to start.

Task 10: Testing of dual foam in swine noncompressible model (closed penetrating wound).

[UMM]

This task has yet to start. The subcontract with TDMI (the actual facility where the Task 10 experiments were to be performed) was canceled in September of 2014 (see Appendix P). The work of Task 10 will be accomplished at UNMC in Y4.

Task 11: Delivery of report on final recommended product description for dual foam device for treatment of noncompressible hemorrhage. [LNK, UNL, UMM, UNMC]

This task has yet to start.

Task 12: Delivery of resorbable bandage for final preclinical study in hypothermic coagulopathic model. [LNK]

As described in the Y2 Annual Report, difficulties with bandage adhesion to the wound interface within the hypothermic coagulopathic subject were encountered, and so animal experimentation in this Task was suspended. The strategy was to perform extensive in vitro work to optimize the adhesion phenomena between the synthetic bandage/fibrin sealant device and the surface of liver, in the absence of endogenous clotting factors. Good quantitative experimentation for this purpose would require a tensiometer, with sensitivity down to 0.1 gram-force [*5*]. Toward this end, in Y3 the project PI applied for and obtained DURIP funding to purchase a $50K Instron tensiometer (see Appendix O). This instrument will be delivered to the PI's lab in November 2014, at which time the intended *in vitro* adhesion experimentation can commence. After the bandage adhesion phenomena can be optimized, then *in vivo* testing for Task 12 will resume.

Task 13: Delivery of fibrin sealant for final preclinical study in hypothermic coagulopathic model. [UNL]

We will continue to generate fibrin sealant so that Tasks 12, 14, and 15 can be completed.

Task 14: Final preclinical study of resorbable bandage for the hypothermic coagulopathic model (swine). [UNMC]

See discussion under Task 12.

Task 15: Delivery of report on final recommended product description for resorbable fibrin

sealant bandage for treatment of compressible coagulopathic hemorrhage. [LNK, UNL, UNMC]

The task has yet to start.

4. KEY RESEARCH ACCOMPLISHMENTS

Bulleted list of key research accomplishments emanating from this research. Project milestones, such as simply completing proposed experiments, are not acceptable as key research accomplishments. Key research accomplishments are those that have contributed to the major goals and objectives and that have potential impact on the research field. If there is nothing to report, simply state "Nothing to report."
[from the USAMRMC instructions:
mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

- Development of a model of severe noncompressible intraabdominal hemorrhage

- Development of a composite alginate/fibrin sealant foam for the treatment of noncompressible intraabdominal hemorrhage

- Development of a device to deliver a composite alginate/fibrin sealant foam

5. CONCLUSION

Summarize the importance and/or implications with respect to medical and /or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.
[from the USAMRMC instructions:
mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

This overall goal of this project is to develop hemostatic technology for two severe clinical scenarios: (1) noncompressible (truncal) hemorrhage, which is the topic of Aim 1 of the project proposal; and (2) solid organ hemorrhage in a cold coagulopathic subject, which is the topic of Aim 2. Conclusions drawn from the work during the third year of this project are as follows:

1. Biologic materials have been produced in sufficient quantities to continue research and development.

2. A porcine noncompressible model of torso hemorrhage involving a hepatic laceration with open technique can produce a lethal and reproducible endpoint.

3. Our studies of the dual foam technology have produced a device capable of delivering a liquid fibrin sealant coated carrier foam into the abdominal cavity of a swine.

4. Initial studies with the dual (alginate/fibrin sealant) foam in the noncompressible hemorrhage model did demonstrate increased efficacy over the no treatment controls. It was hypothesized that the lack of effect was secondary to the high fluid administration rate; subsequent off-protocol studies demonstrated that high-fluid administration did indeed contribute to poor outcome after the noncompressible injury mechanism. We will modify our animal protocol accordingly to allow for a decreased fluid administration rate, and repeat the foam efficacy studies.

5. Continued engineering of the foam carrier technology is needed to increase efficacy of the foam treatments in the noncompressible model. On a related note, we have introduced a second form of the noncompressible model, involving nonanatomic transection of the left medial lobe (currently under ACURO review), in order to provide a more varied testing background for the foam technology.

6. The bandage-wound adhesion phenomenon in the hypothermic hemodiluted porcine subject will need extensive *ex vivo* study in order to develop an efficacious bandage whose efficacy does not depend on gram quantities of fibrinogen. This testing will commence after the delivery of the high-precision tensiometer, which was funded by a DURIP proposal submitted during Y3.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

> *1. Lay Press:*
> *2. Peer-Reviewed Scientific Journals:*
> *3. Invited Articles:*
> *4. Abstracts:*

b. List presentations made during the last year (international, national, local societies, military meetings, etc.).

Use an asterisk (*) if presentation produced a manuscript.
[from the USAMRMC instructions:
mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

a. Publications

a1. Lay Press: nothing to report.

a2. Peer Reviewed Scientific Journals:

1. Carlson MA, Calcaterra J, Johanning JM, Pipinos, II, Cordes CM, Velander WH. A totally recombinant human fibrin sealant. *J Surg Res* 2014; 187(1): 334-42. See Appendix E.

2. Yanala UR, Johanning JM, Pipinos II, Larsen G, Velander WH, Carlson MA. Development of a fatal noncompressible truncal hemorrhage model with combined hepatic and portal venous injury in normothermic normovolemic Swine. *PLoS ONE* 2014; 9(9): e108293. See Appendix F.

3. Yanala UR, Larsen G, Velander WH, Carlson MA. A synthetic resorbable mesh for minor surgical hemorrhage. Manuscript in preparation (off protocol).

4. Yanala UR, Johanning JM, Pipinos II, Larsen G, Velander WH, Carlson MA. Synthetic resorbable bandages with liquid fibrin sealant for hepatic resection procedures in swine. Manuscript in preparation (off protocol).

a3. Invited Articles: nothing to report.

a4. Abstracts:

1. Yanala UR, Johanning JM, Pipinos II, Velander WH, Carlson MA. Development of a porcine model of severe noncompressible truncal hemorrhage. *J Surg Res* 2014;186:510. Presented at the 2014 meeting of Academic Surgical Congress; the See Appendices G and R.

2. Yanala UR, Noriega S, Spretz R, Ragusa J, Nuñez L, Larsen G, Carlson MA. Synthetic Resorbable vs. Cellulose Bandage for Minor Hemorrhage in a Porcine Model. Submitted to the 2015 meeting of the Academic Surgical Congress. See Appendix H; off-protocol.

b. Presentations

1. "Development of a porcine model of severe noncompressible truncal hemorrhage"
   Yanala UR, Johanning JM, Pipinos II, Velander WH, Carlson MA
   2014 Academic Surgical Congress
   San Diego, CA. February, 2014
   See Appendix R

2. "Development of a Model of Internal Bleeding" [poster]
   Yanala UR, Johanning JM, Pipinos II, Velander WH, Carlson MA
   2014 Omaha VA Medical Center Research Week
   Omaha, NE. May, 2014
   See Appendix N

3. "Development of a porcine model of severe noncompressible truncal hemorrhage"
   [poster]
   Carlson MA, Yanala UR, Johanning JM, Pipinos II, Velander WH
   2014 Swine in Biomedical Research Conference
   Raleigh, NC. July, 2014
   See Appendix N

4. Annual presentation to project scientific officers at the ISR
   Carlson MA
   San Antonio, TX. May, 2014
   See Appendix I

5. Annual presentation to project scientific officers at the ISR
   Velander WH
   San Antonio, TX. May, 2014
   See Appendix J

6.   Vanderslice N, Calcaterra J, Ismail A, Fatemi M, Hansen BC, Cordes CM, Heimann D, Yanala UR, Spretz R, Larsen G, Nuñez L, Carlson MA, Velander WH.
       "Treatment of Hepatic Resection in Swine using Novel Delivery Methods for Fibrin Sealant" [poster and presentation; off-protocol]
       2014 Swine in Biomedical Research Conference
       Raleigh, NC. July, 2014
       See Appendices K and Q

7. INVENTIONS, PATENTS, AND LICENSES

List all patents and licenses applied for and/or issued. Each entry must include the inventor(s), invention title, patent application number, filing date, patent number if issued, patent issued date, national, or international.
[from the USAMRMC instructions:
mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

Nothing to report.

8. REPORTABLE OUTCOMES

Provide a list of reportable products that have resulted from this research. Products are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. This list may include development of prototypes or similar products that may be commercialized. [from the USAMRMC instructions: mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

1. Development of a model of severe noncompressible intraabdominal hemorrhage

2. Development of a composite alginate/fibrin sealant foam for the treatment of noncompressible intraabdominal hemorrhage

3. Development of a device to deliver a composite alginate/fibrin sealant foam

9. OTHER ACHIEVEMENTS

This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.
[from the USAMRMC instructions:
mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting_pre_FY14]

In addition to the above, the project investigators have been proceeding with Phase 2 of a Program from the Department of Economic Development of the State of Nebraska for a separate (**not** funded by the DoD) but related project entitled "Hemostatic Patch," which studied elective surgical applications for the synthetic resorbable mesh devices. The Phase 1 component ($100,000 of funding) has been completed; see the abstract in Appendix H for a preliminary summary of results (a manuscript on this topic also is in preparation). Phase 2 funding ($400,000) was approved in February 2014 (see Appendix T); the goal of this work will be to develop fibrin sealant-synthetic bandage composites for use in hepatic resection procedures and other elective operations.

REFERENCES

1.   Yanala UR, Johanning JM, Pipinos, II, Larsen G, Velander WH, Carlson MA. Development of a fatal noncompressible truncal hemorrhage model with combined hepatic and portal venous injury in normothermic normovolemic Swine. *PLoS ONE* 2014; 9(9): e108293.
2.   Duggan M, Rago A, Sharma U, Zugates G, Freyman T, Busold R, et al. Self-expanding polyurethane polymer improves survival in a model of noncompressible massive abdominal hemorrhage. *J Trauma Acute Care Surg* 2013; 74(6): 1462-7.
3.   Langan NR, Eckert M, Martin MJ. Changing Patterns of In-Hospital Deaths Following Implementation of Damage Control Resuscitation Practices in US Forward Military Treatment Facilities. *JAMA Surg* 2014; 149(9): 904-12.
4.   Champion HR, McSwain NE, Richard B, Weiskopf M. Fluid Resuscitation for Hemorrhagic Shock in Tactical Combat Casualty Care. *J Spec Op Med* 2014; 14(3): 30-55.
5.   Carlson MA, Chakkalakal D. Tensile properties of the murine ventral vertical midline incision. *PLoS ONE* 2011; 6(9): e24212.

11. APPENDICES

List of Appendices

Appendix A (Gantt Chart), for Y3Q4 Annual Report, October 25, 2014

Project Title: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"
Award Number: W81XWH-11-1-0836
Grant Number: 10091006
PI: Carlson, Mark A.
Reporting period: 09-27-2013 to 09-26-2014 (Y3 Annual Report)
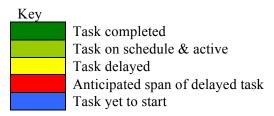
Appendix A. Gantt Chart (current quarter in boldface). See Appendix B for definitions of Tasks.

Task	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4	Y3Q1	Y3Q2	Y3Q3	**Y3Q4**	Y4Q1	Y4Q2	Y4Q3	Y4Q4	Status
1	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
2	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
3	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
4					yellow	green	green	green	green	green	green	green					Delayed
5	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
6	yellow	yellow	yellow	yellow	yellow	green	green	green	green	green	green	green	red	red	red		Delayed
7					yellow	green	green	green	green	green	green	green					Delayed
8									yellow	yellow	blue	blue	blue	blue	blue	red	Delayed
9								green	green	green	green	green					On schedule
10									yellow	yellow	blue	blue	red	red			Delayed
11													blue	blue	blue	blue	Yet to start
12	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
13	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
14	green	green	green	green	green	green	green	green	green	green	green	green					On schedule
15					yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow	red	red			Delayed

Key

dark green	Task completed
green	Task on schedule & active
yellow	Task delayed
red	Anticipated span of delayed task
blue	Task yet to start

Appendix B

Proposal Title: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"
USAMRMC No. 10091006
Contract No. W81XWH-11-1-0836
PI: Carlson, Mark A.
SOW version date: February 13, 2013

STATEMENT OF WORK

No.	Task description	Site	Year	Aim
1	Purification/generation of pd-FI and rFXIIIA2-a	UNL	1-3	1
2	Generation of ultrafine particles for tamponade carrier foam	LNK	1-3	1
3	Testing of candidate tamponade carrier & FS foams	LNK & UNL	1-2	1
4	Testing of single foams in swine (tamponade carrier & FS foams separately)	UNMC	2-3	1
5	Development & engineering of dual foam candidate devices	LNK & UNL	1-2	1
6	Testing of dual foam in swine	UNMC	2-3	1
7	Engineering of firm foams from alginate and alginate derivatives	LNK	2-3	1
8	Testing of dual foam in swine noncompressible model (laparotomy with 2° closure)	UNMC	3-4	1
9	Delivery of candidate field-ready dual foam device	UNL & LNK	3	1
10	Testing of dual foam in swine noncompressible model (closed penetrating wound)	UMM	3	1
11	Delivery of report on final recommended product description for dual foam device for treatment of noncompressible hemorrhage	LNK, UNL, UNMC, UMM	4	1
12	Delivery of resorbable bandage for final preclinical study in hypothermic coagulopathic model	LNK	1	2
13	Delivery of fibrin sealant for final preclinical study in hypothermic coagulopathic model	UNL	1	2
14	Final preclinical study of resorbable bandage in for hypothermic coagulopathic model (swine)	UNMC	1	2
15	Delivery of report on final recommended product description for resorbable fibrin sealant bandage for treatment of compressible coagulopathic hemorrhage	LNK, UNL, UNMC	2	2

List of Abbreviations

ACURO	Animal Care and Use Review Office
DCR	damage control resuscitation
DI	deionized
DoD	Department of Defense
DURIP	Defense University Research Instrumentation Program
EHD	electrohydrodynamics
ETCO2	end tidal carbon dioxide
FI	Factor I (fibrinogen)
FIIa	activated Factor II (thrombin)
FN	fibronectin
FS	fibrin sealant
FXIII	Factor XIII (cross-linking factor)
Hb	hemoglobin
HPC	hydroxypropylcellulose
HPSEC	High pressure size exclusion chromatography
IACUC	Institutional Animal Care and Use Committee
ISR	Institute of Surgical Research
IVC	inferior vena cava
IVF	intravenous fluids
LFS	Liquid Fibrin Sealant
LNK	LNKChemsolutions, LLC
LR	Lactated Ringers solution
MAP	mean arterial pressure
PCL	polycaprolactone
pd	plasma derived
PLA	polylactic acid
PT	protime
rFXIIIA2-a	activated recombinant Factor XIII
SBF	simulated body fluid
SDS PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	scanning electron microscopy
SOW	Statement of Work
TCCC	Tactical Combat Casualty Care
TDMI	Thomas D. Morris Institute
TEG	thromboelastography
UMM	University of Maryland Medical Center
UNL	University of Nebraska—Lincoln
UNMC	University of Nebraska Medical Center

I. OVERVIEW

Date: October 1, 2013
Swine no: 181 (ear tag no. 303355)
Model: swine, normothermic, normovolemic small liver excision
Treatment: PCL
Survival: YES
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega

II. PRE-INJURY PHASE

Start time (induction): 7:45 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 09/27/2013
Pre-procedure wt: 34.5 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane
Fentanyl path (100 µg/h) applied at 8:05 AM
Buprenorphine (1 mL = 0.3 mg IM 8:57 AM)

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for IVF
4. Rectal temp probe
5. Pulse oximetry
6. Heating pad below subject

Initial VS
- HR: 141
- Temp: 38.0
- O2 Sat: 99
- ETCO2: 39

Blood draw no. 1 (only one; left jugular vein stick): 7:55 AM (CBC, Chem 20, amylase/lipase, PT/PTT/INR, fibrinogen, TEG)

Antibiotics: 8 mg/kg Cefovecin IM at 7:52 AM

Pre-injury VS
- HR: 137
- Temp: 36.8
- O2 Sat: 96
- ETCO2: 36

III. INJURY & TREATMENT PHASE

Skin incision: 8:13 AM
Time of injury: 8:21 AM
Injury type: Small liver excision. Under sterile conditions, a ventral midline incision (~11 cm in length) was created, and a 6 cm strip of liver (wt = 1.89 g) was excised from the edge of the left medial lobe (see Figures).
Treatment description: PCL. Final folded dimensions ~6.5 x 3.5 cm (see Figures). Wt = 0.35 g.
Clotting factors: none.
Technique: the liver strip was excised with cold scissors, and the PCL was applied immediately to the wound with manual pressure. After a period of 4 min, hemostasis was complete (see Figures).
Abdominal closure: Immediately after hemostasis was obtained, the midline incision was closed with 0-Maxon (running mass closure), followed by 4-0 Vicryl subcuticular.
Blood loss during procedure: 6 mL
Duration of procedure (beginning of incision to completion of skin closure): 42 min.
Amount LR infused: 1000 mL

Post-closure VS
- HR: 126
- Temp: 37.1
- O2 Sat: 97
- ETCO2: 33

IV. RECOVERY PHASE

Anesthetic gas (running at 0.5%) was shut off during the wound closure (8:30 AM). Animal extubated without incident at 9:15 AM, and recovered without difficulty.

V. COMMENTS

Subject no. 9 in the swine survival trial of Surgicel® vs. PCL gauze in the treatment of a small hepatic excision. The endpoints of this 28-day survival trial will address toxicity issues. Treated with PCL per the randomization schedule. Went well, no issues with this subject.

VI. PLAN

Continue protocol per randomization schedule. Total N will be 12.

Figure 1, Swine 181. Preparation for injury. Left medial lobe of liver (LM) has been exteriorized through short ventral midline incision. Extent of excision has been scored on liver capsule with cautery (arrows). Cephalad is to the left.
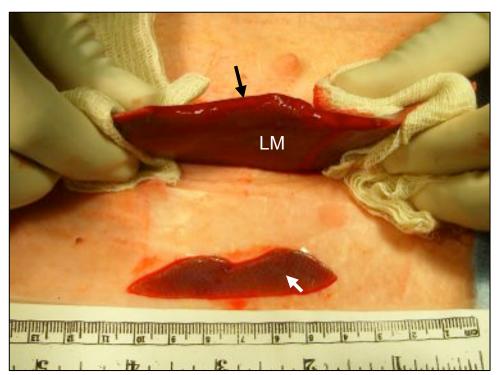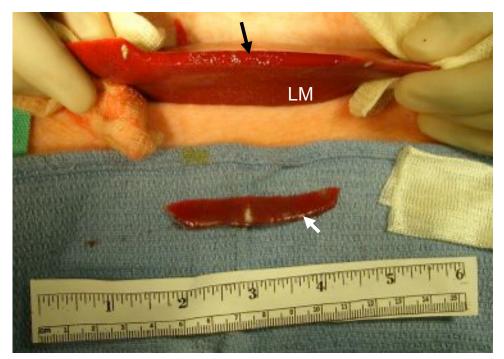

Figure 2, Swine 181. Post-excision. Excised strip is in foreground (white arrow). Oozing edge of excisional injury on LM lobe indicated with black arrow. Cephalad is to the left.
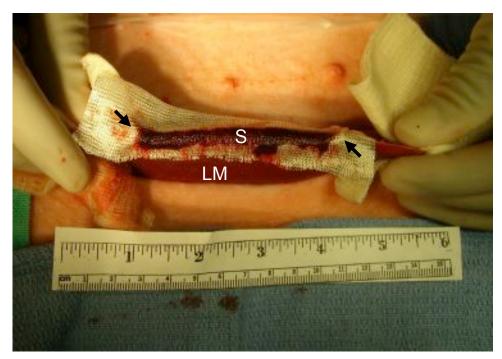
Swine 181, Figures, 1 of 2
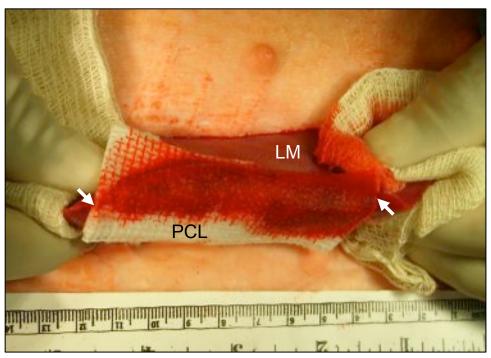
Figure 3, Swine 181. Post-treatment. PCL shown covering wound on LM lobe. Ends of wound indicated with arrows. Cephalad is to the left.

I. OVERVIEW
Date: October 1, 2013
Swine no: 182 (ear tag no. 303367)
Model: swine, normothermic, normovolemic small liver excision
Treatment: Surgicel
Survival: YES
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega

II. PRE-INJURY PHASE

Start time (induction): 9:20 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 09/27/2013
Pre-procedure wt: 34.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane
Fentanyl path (100 μg/h) applied at 9:30 AM
Buprenorphine (1 mL = 0.3 mg IM 10:38 AM)

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for IVF
4. Rectal temp probe
5. Pulse oximetry
6. Heating pad below subject

Initial VS
- HR: 177
- Temp: 38.6
- O2 Sat: 99
- ETCO2: 44

Blood draw no. 1 (only one; left jugular vein stick): 9:36 AM (CBC, Chem 20, amylase/lipase, PT/PTT/INR, fibrinogen, TEG)

Antibiotics: 8 mg/kg Cefovecin IM at 9:38 AM

Pre-injury VS
- HR: 189
- Temp: 38.6
- O2 Sat: 97
- ETCO2: 39

III. INJURY & TREATMENT PHASE

Skin incision: 9:53 AM
Time of injury: 9:59 AM
Injury type: Small liver excision. Under sterile conditions, a ventral midline incision (~11 cm in length) was created, and a 6 cm strip of liver (wt = 1.75 g) was excised from the edge of the left medial lobe (see Figures).
Treatment description: Surgicel. Final folded dimensions ~9.5 x 3.5 cm (see Figures). Wt = 0.34 g.
Clotting factors: none.
Technique: the liver strip was excised with cold scissors, and the Surgicel was applied immediately to the wound with manual pressure. After a period of 1 min, hemostasis was complete (see Figures).
Abdominal closure: Immediately after hemostasis was obtained, the midline incision was closed with 0-Maxon (running mass closure), followed by 4-0 Vicryl subcuticular.
Blood loss during procedure: 1 mL
Duration of procedure (beginning of incision to completion of skin closure): 42 min.
Amount LR infused: 1000 mL

Post-closure VS
- HR: 135
- Temp: 38.5
- O2 Sat: 96
- ETCO2: 36

IV. RECOVERY PHASE

Anesthetic gas (running at 0.5%) was shut off during the wound closure (10:09 AM). Animal extubated without incident at 10:51 AM, and recovered without difficulty.

V. COMMENTS

Subject no. 10 in the swine survival trial of Surgicel® vs. PCL gauze in the treatment of a small hepatic excision. The endpoints of this 28-day survival trial will address toxicity issues. Treated with Surgicel per the randomization schedule. Subject did well; no issues today.

VI. PLAN

Continue protocol per randomization schedule. Total N will be 12. Two swine from today will be sacrificed Oct 29th. Next swine procedures (last two for this protocol) will be two weeks from today, on Tue Oct. 15th.

Figure 1, Swine 182. Surgicel® (J&J, no. 1952). A 4 x 8 in section has been cut into 1/3 and 2/3 sized pieces. The 1/3 sized piece was folded along the dotted line and used in this subject.
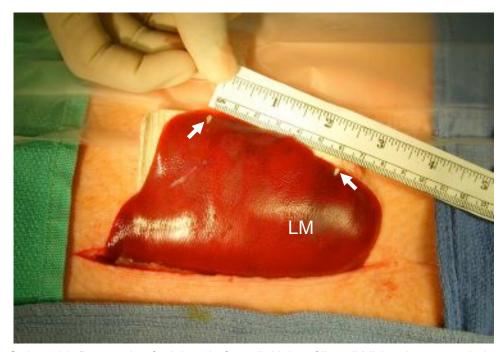

Figure 2, Swine 182. Preparation for injury. Left medial lobe of liver (LM) has been exteriorized through short ventral midline incision. Extent of excision has been scored on liver capsule with cautery (arrows). Cephalad is to the left.
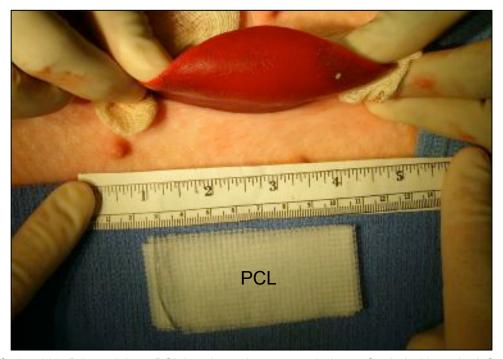
Figure 3, Swine 182. Post-excision. Excised strip is in foreground (white arrow). Oozing edge of excisional injury on LM lobe indicated with black arrow. Cephalad is to the left.

Figure 4, Swine 182. Post-treatment. Surgicel (S) shown covering wound on LM lobe. Ends of wound indicated with arrows. Cephalad is to the left.

I. OVERVIEW
Date: October 15, 2013
Swine no: 183 (ear tag no. 304085)
Model: swine, normothermic, normovolemic small liver excision
Treatment: PCL
Survival: YES
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega

II. PRE-INJURY PHASE

Start time (induction): 7:55 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/10/2013
Pre-procedure wt: 38.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane
Fentanyl path (100 μg/h) applied at 8:10 AM
Buprenorphine (1 mL = 0.3 mg IM 9:26 AM)

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for IVF
4. Rectal temp probe
5. Pulse oximetry
6. Heating pad below subject

Initial VS
- HR: 130
- Temp: 38.0
- O2 Sat: 98
- ETCO2: 30

Blood draw no. 1 (only one; left jugular vein stick): 8:30 AM (CBC, Chem 20, amylase/lipase, PT/PTT/INR, fibrinogen, TEG)

Antibiotics: 8 mg/kg Cefovecin IM at 8:05 AM

Pre-injury VS
- HR: 109
- Temp: 38.1
- O2 Sat: 95
- ETCO2: 24

III. INJURY & TREATMENT PHASE

Skin incision: 8:38 AM

Time of injury: 8:45 AM

Injury type: Small liver excision. Under sterile conditions, a ventral midline incision (~11 cm in length) was created, and a 6 cm strip of liver (wt = 1.88 g) was excised from the edge of the left medial lobe (see Figures).

Treatment description: PCL. Final folded dimensions ~8.0 x 3.5 cm (see Figures). Wt = 0.27 g.

Clotting factors: none.

Technique: the liver strip was excised with cold scissors, and the PCL was applied immediately to the wound with manual pressure. After a period of 3 min, hemostasis was complete.

Abdominal closure: Immediately after hemostasis was obtained, the peritoneal layer was closed with running 3-0 Vicryl, the linea alba was closed with 0-Maxon (running mass closure), and the skin was closed with 4-0 Vicryl subcuticular.

Blood loss during procedure: 6 mL

Duration of procedure (beginning of incision to completion of skin closure): 45 min.

Amount LR infused: 730 mL

Post-closure VS
- HR: 82
- Temp: 37.2
- O2 Sat: 97
- ETCO2: 24

IV. RECOVERY PHASE

Anesthetic gas (running at 0.5%) was shut off during the wound closure (8:58 AM). Animal extubated without incident at 9:33 AM, and recovered without difficulty.

V. COMMENTS

Subject no. 11 in the swine survival trial of Surgicel® vs. PCL gauze in the treatment of a small hepatic excision. The endpoints of this 28-day survival trial will address toxicity issues. Treated with PCL per the randomization schedule. Went well, no issues with this subject.

VI. PLAN

Continue protocol per randomization schedule. Total N will be 12.
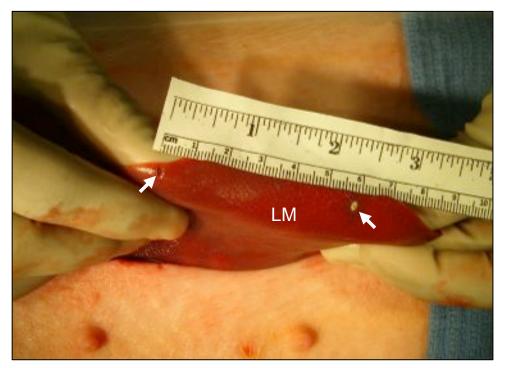
Figure 1, Swine 183. Preparation for injury. Left medial lobe of liver (LM) has been exteriorized through short ventral midline incision. Extent of excision has been scored on liver capsule with cautery (arrows). Cephalad is to the left.

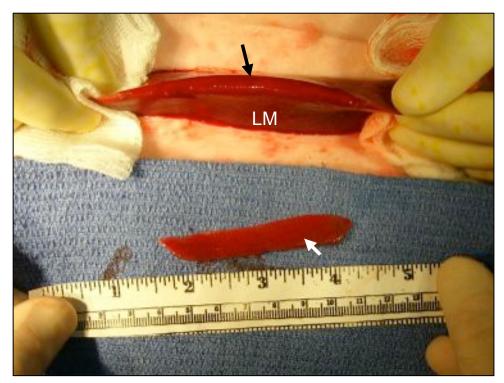Figure 2, Swine 183. Prior to injury; PCL bandage shown; wt = 270 mg. Cephalad is to the left.

Figure 3, Swine 183. Post-excision. Excised strip is in foreground (white arrow). Oozing edge of excisional injury on LM lobe indicated with black arrow. Cephalad is to the left.

I. OVERVIEW
Date: October 15, 2013
Swine no: 184 (ear tag no. 304090)
Model: swine, normothermic, normovolemic small liver excision
Treatment: PCL
Survival: YES
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega

II. PRE-INJURY PHASE

Start time (induction): 9:38 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/10/2013
Pre-procedure wt: 36.8 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane
Fentanyl path (100 µg/h) applied at 9:49 AM
Buprenorphine (1 mL = 0.3 mg IM 11:10 AM)

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for IVF
4. Rectal temp probe
5. Pulse oximetry
6. Heating pad below subject

Initial VS
• HR: 109
• Temp: 39.0
• O2 Sat: 98
• ETCO2: 36

Blood draw no. 1 (only one; left jugular vein stick): 9:59 AM (CBC, Chem 20, amylase/lipase, PT/PTT/INR, fibrinogen, TEG)

Antibiotics: 8 mg/kg Cefovecin IM at 9:47 AM

Pre-injury VS
• HR: 120
• Temp: 38.5
• O2 Sat: 92
• ETCO2: 29

III. INJURY & TREATMENT PHASE

Skin incision: 10:12 AM
Time of injury: 10:18 AM
Injury type: Small liver excision. Under sterile conditions, a ventral midline incision (~11 cm in length) was created, and a 6 cm strip of liver (wt = 1.86 g) was excised from the edge of the left medial lobe (see Figures).
Treatment description: PCL. Final folded dimensions ~8.0 x 3.5 cm (see Figures). Wt = 0.26 g.
Clotting factors: none.
Technique: the liver strip was excised with cold scissors, and the PCL was applied immediately to the wound with manual pressure. After a period of 1 min, hemostasis was complete.
Abdominal closure: Immediately after hemostasis was obtained, the peritoneal layer was closed with running 3-0 Vicryl, the linea alba was closed with 0-Maxon (running mass closure), and the skin was closed with 4-0 Vicryl subcuticular.
Blood loss during procedure: 2 mL
Duration of procedure (beginning of incision to completion of skin closure): 35 min.
Amount LR infused: 1000 mL

Post-closure VS
- HR: 117
- Temp: 38.0
- O2 Sat: 96
- ETCO2: 27

IV. RECOVERY PHASE

Anesthetic gas (running at 0.5%) was shut off during the wound closure (10:29 AM). Animal extubated without incident at 11:11 AM, and recovered without difficulty.

V. COMMENTS

Subject no. 12 in the swine survival trial of Surgicel® vs. PCL gauze in the treatment of a small hepatic excision. The endpoints of this 28-day survival trial will address toxicity issues. Treated with PCL per the randomization schedule. Went well, no issues with this subject.

VI. PLAN

This was the animal to be wounded in the NEDED survival trial. Necropsy of today's subjects will be on Tue Nov 12th.
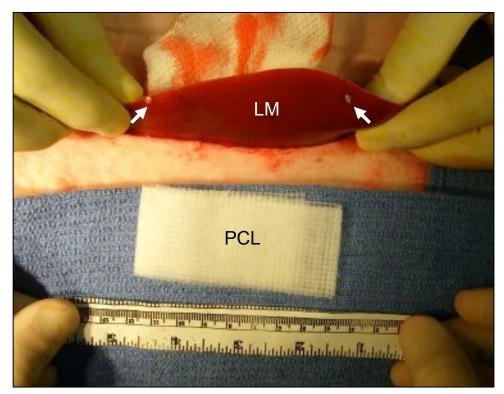
Figure 1, Swine 184. Preparation for injury. Left medial lobe of liver (LM) has been exteriorized through short ventral midline incision. Extent of excision has been scored on liver capsule with cautery (arrows); PCL bandage (260 mg) to be used shown in the foreground. Cephalad is to the left.

Figure 2, Swine 184. Post-excision. Excised strip is in foreground (white arrow). Oozing edge of excisional injury on LM lobe indicated with black arrow. Cephalad is to the left.
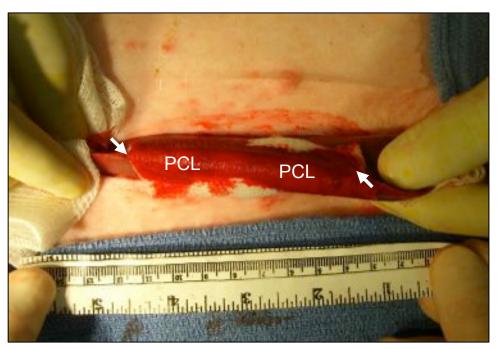
Figure 3, Swine 184. Post-treatment. PCL shown covering wound on LM lobe. Ends of wound indicated with arrows. Cephalad is to the left.

I. OVERVIEW
Date: October 22, 2013
Swine no: 185
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: Calcium alginate foam (new formulation) without dye; no biologics
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega, Fatemi, Vanderslice, Ismail

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II. PRE-INJURY PHASE

Start time: 8:06 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/18/2013
Pre-procedure wt: 37.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 115
- MAP: 90
- Temp: 37.4

Blood draw no. 1 (initial): 8:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:32 AM
Spleen wt: 341 gm
LR (22°C) infused after splenectomy: 1025 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 341 + 20 + 100 = 461 mL
- LR (22°C) infused (spleen replacement + incidental): 1025 + 330 = 1355 mL

Pre-injury VS
- HR: 97
- MAP: 92
- Temp: 35.8

III. INJURY & TREATMENT PHASE

Time of injury: 8:58 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: calcium alginate foam without dye and without biologics. Different source ("High G Block" alginate). 0.6 M calcium chloride (syringe pump, ~100 mL), no xanthum gum, contains 0.87% Tween (essential for foaming); same creamer dispenser-based delivery system. Initial foam volume ~5 L. Foam injection begun 30 seconds after injury.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began ~30 sec after injury, after the abdomen had been completely closed with clips. After injection completed (~3 min after injury), the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Abdomen became distended during injection.

IAP: not recording accurately at the beginning, but began working after a few minutes. Max reading was 19 toward end of procedure, and 16 upon completion.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 72

Resuscitation fluid: warm LR (3.7 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 8:59 (with 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 85
- MAP: 66
- Temp: 35.7

Blood draw no. 3: (60 min post-injury): 9:58 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (60 min) VS
- HR: 73
- MAP: 39
- Temp: 36.2

Survival at 60 min? Yes
Target MAP attained? multiple times
Time of death: 10:05 AM
Cause of death: intentional exsanguination from IVC transection

Interval from injury to death: 67 min

Post-treatment fluid data:
- Blood loss (intraabdominal suctioning + pads/gauze + phlebotomy):) 1380 + 580 + 20 mL = 1980* mL
- IV fluid given: LR (37°C): 4640 mL

*About 150 g of blood loss was attributable to foam mass.

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, with IAP = 16 mm Hg. No butane gas pocket
when incision reopened. Foam covering intestines on right side of abdomen. The foam initially was white
when the abdomen was re-opened, but became stained with blood as we proceeded to expose it more (see
Figures). The foam was not mixed with blood. Volume of foam retrieved = 500 mL; see Figures. Clotted
blood not mixed with foam in deeper areas of abdomen. The intestines were discolored purplish, but not as
severe as with previous iterations of foam.
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe
Portal vein injury: 1 branch, to LL lobe
Other: none.
Ex vivo total liver wt: 829 g.

Tissue harvested: none

VI. COMMENTS

        New iteration of calcium alginate foam tested in noncompressible model. This alginate was from a
different source. Calcium concentration reduced from ~2 M to 0.6 M. Residual foam volume after 1 h *in vivo* was
500 mL, or 10% of the approximate starting volume (~5 L). Minimal mixing of blood with foam. Foam did seem
somewhat less toxic to intestine. Animal survived the 1 h observation.
        I think we need a more "durable" foam, i.e., a foam that can retain most of the starting volume after
sitting within the abdomen for an hour. Also, I'm not sure if we have solved the calcium toxicity issue… this still
seems to be there, if decreased. If we show efficacy with these nonsurvival studies, then we definitely will need to
determine if the Ca alginate foam has toxicity over a 30 day observation period in a swine survival study.

VII. PLAN

        Repeat above testing next week. Mostafa said he will try to decrease the Tween in the foam, which
currently is ~0.9%.
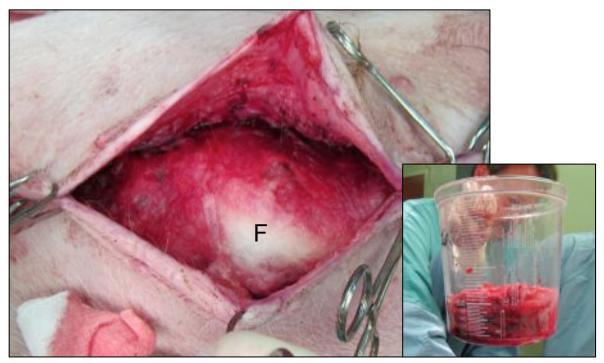
Figure 1, swine 185. Overhead view of the abdomen immediately after 1 hr observation period; swine survived. Midline incision reopened; foam (F) shown within abdomen (final volume removed ~500 mL; see inset). Cephalad is to the left.
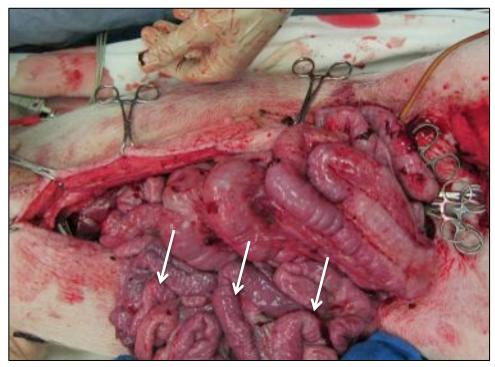
Figure 2, swine 185. Overhead view of the abdomen after observation period & removal of foam/blood. The intestines appear somewhat dusky (arrows) Cephalad is to the left.
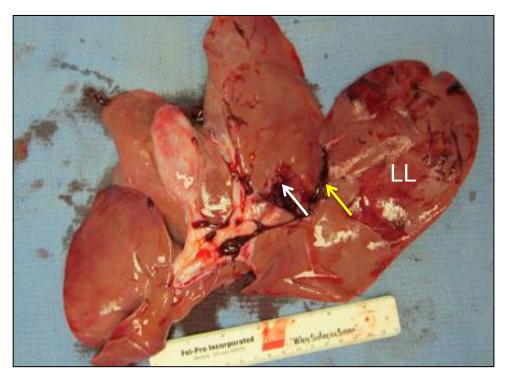
Figure 3, swine 185. Liver *ex vivo,* inferior aspect. Transected HV to LL lobe shown with white arrow. Transected branch of PV to LL lobe shown with yellow arrow.

I. OVERVIEW
Date: October 22, 2013
Swine no: 186
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: Barbasol foam + all three biologics
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega, Fatemi, Vanderslice, Ismail

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II. PRE-INJURY PHASE

Start time: 10:36 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/18/2013
Pre-procedure wt: 36.4 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 102
- MAP: 113
- Temp: 40.5

Blood draw no. 1 (initial): 10:45 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:56 AM
Spleen wt: 320 gm
LR (22°C) infused after splenectomy: 960 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 320 + 20 + 21 = 361 mL
- LR (22°C) infused (spleen replacement + incidental): 960 + 200 = 1160 mL

Pre-injury VS
- HR: 95
- MAP: 108
- Temp: 38.2

III. INJURY & TREATMENT PHASE

Time of injury: 11:06 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: Barbasol foam with pdFI (22.5 mg/mL), rFIIa (106 U/mL), and rFXIIIa (0.72 mg/mL).

Clotting factors: all three.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began ~30 sec after injury, after the abdomen had been completely closed with clips. After injection completed (~1 min after injury), the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Abdomen became distended during injection.

IAP: Max reading was 16, toward end of procedure.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 87

Resuscitation fluid: warm LR (3.6 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 11:08 (with 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 129
- MAP: 42
- Temp: 37.8

Blood draw no. 3: (60 min post-injury): 12:04 PM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (58 min) VS
- HR: 75
- MAP: 12
- Temp: 36.7

Survival at 60 min? No
Target MAP attained? No
Time of death: 12:05 PM
Cause of death: exsanguination from liver injury
Interval from injury to death: 59 min

Post-treatment fluid data:
- Blood loss (intraabdominal suctioning + pads/gauze + phlebotomy):) 2288 + 1401 + 20 mL = 3709 mL
- IV fluid given: LR (37°C): 3900 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, with IAP = 15-16 mm Hg. No gas pocket when incision reopened. Two clips removed and Barbasol foam, white, was extruded through incision with manual pressure (see Figures). Most of foam removed from abdomen in this manner; most of the foam was white. All clips then removed, and incision completely re-opened. Remaining foam was mixing with unclotted blood, forming a "soup" that was bathing the intestines. No clotted blood was present, except in deep recesses of abdomen, where there was no foam (see Figures). Could not detect obvious evidence of intestinal toxicity, though intestines were covered with the "soup."

Heart: not examined.

Number of hepatic veins lacerated: large injury to confluence of HVs to LM & LL lobes (see Figs)

Portal vein injury: 2 branches, to LL lobe

Other: clot found in suprahepatic IVC

Ex vivo total liver wt: 965 g.

Tissue harvested: none

VI. COMMENTS

Barbasol-based foam + biologics in noncompressible model; [pdFI] in this formulation was >2x the previous formulation. No effect of biologics/foam evident. All clot appeared to be in areas not reached by foam (i.e., deep recesses). Does the Barbasol foam inhibit clotting *in vivo?*

After observing the effects of various foam treatments in this noncompressible model for ~20 swine, I am thinking that the main determinant of efficacy in this model will be the mechanical compression/tamponade provided by the foam. Today's results (both no. 185 & 186) reinforced the fact that in this noncompressible model, very little mixing occurs between the injected foam and the subject's blood. In fact, the foam (whether alginate or shaving cream) appears to displace the intraperitoneal blood without interacting with it. As a result, very little if any clotting appears to result from the foam injection. If we presume that the biologics will need to mix with the subject's blood in order to increase clotting, and since it appears to be quite difficult to obtain adequate mixing of injected foam with the ongoing hemorrhage, I'm not sure what contribution of biologics can be in this model. So in order to get a treatment for noncompressible hemorrhage with some demonstrable efficacy, I propose that we focus on developing a firm, durable, nontoxic foam.

VII. PLAN

Repeat above testing next week (Tue Oct 29th) for verification.
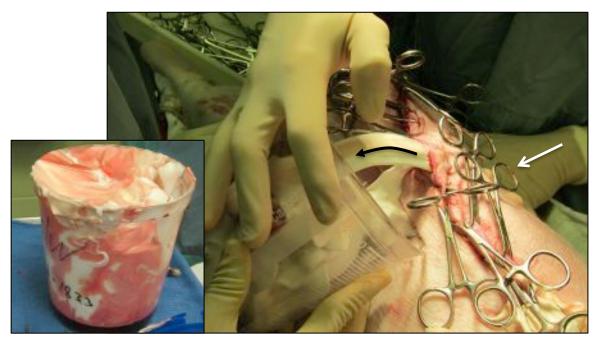
Figure 1, swine 186. Oblique view of abdomen just after expiration. Cephalad is to the lower right. Two clips have been removed from the center of the midline incision. A hand is pushing into the abdomen on the right side (white arrow). This pressure is ejecting a stream of Barbasol foam (black arrow) from the abdomen, through the gap between the clips, and into the bucket. Note absence of blood in the foam. Inset: ejected foam in bucket. Toward the end of the stream, some nonclotted blood mixed with foam (pinkish foam) emerged, giving the swirled appearance.
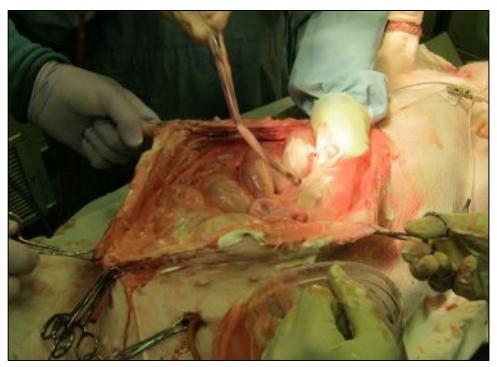
Figure 2, swine 186. Post-expiration. The midline incision has been re-opened. Most of foam has been removed, leaving a "soup" of foam mixed with nonclotted blood that was bathing the intestines. Cephalad is to the upper right.
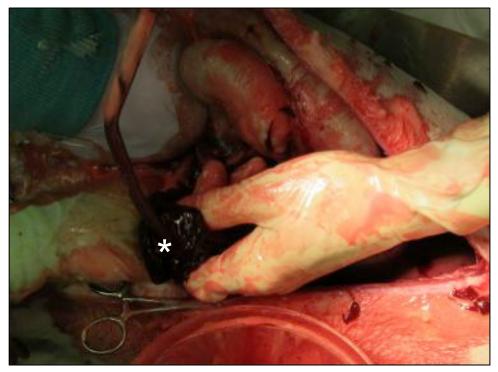
Figure 3, swine 186. View of the abdomen with incision re-opened. After "soup" in Figure 2 was evacuated, some pure red clots (*) not mixed with foam were removed from deep recesses of the abdomen mostly in the subdiaphragmatic regions, in the subhepatic space, and intermingled with the intestines. Cephalad is to the right.
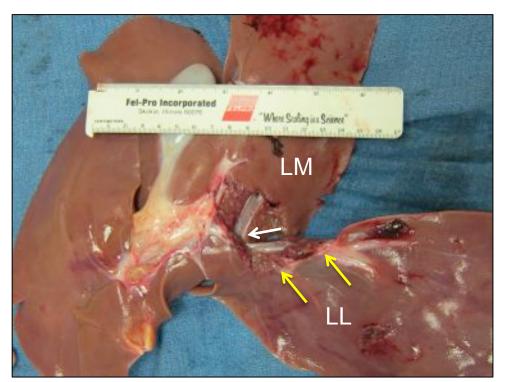
Figure 4, swine 186. Liver *ex vivo,* inferior aspect. Two branches of the PV were transected (yellow arrows). The confluence of the HV to the LL and LM lobes was cut (white arrow), yielding a large venous injury.

I. OVERVIEW
Date: October 29, 2013
Swine no: 187
Model: swine, normothermic, normovolemic noncompressible hemorrhage; non-injury control
Treatment: Calcium alginate foam (new formulation) without dye; no biologics
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega, Fatemi, Vanderslice, Ismail

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II. PRE-INJURY PHASE

Start time: 7:55 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/18/2013
Pre-procedure wt: 38.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 119
- MAP: 125
- Temp: 38.0

Blood draw no. 1 (initial): 8:11 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:25 AM
Spleen wt: 301 gm
LR (22°C) infused after splenectomy: 905 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 301 + 20 + 31 = 352 mL
- LR (22°C) infused (spleen replacement + incidental): 905 + 225 = 1130 mL

Pre-injury VS
- HR: 73
- MAP: 141
- Temp: 34.8

III. INJURY & TREATMENT PHASE

Time of injury: 8:39 AM (foam injection only)

Injury type: no injury. After set-up completed, abdomen towel clip closure, with the nozzle of the foam injector inserted between clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. The line to the IAP monitor exited through the superior end of the midline incision.

Treatment description: calcium alginate foam without dye and without biologics. Different source ("High G Block" alginate). 0.6 M calcium chloride (syringe pump, ~100 mL), no xanthum gum, contains ~0.7% Tween (essential for foaming); same creamer dispenser-based delivery system; 3.25% alginate. Initial foam volume intended to be ~5 L.

Clotting factors: none.

Technique: as above. Injector malfunctioned with leakage of solution from exterior portion apparatus. Very little foam actually was injected into the abdomen, and this probably had an excessively high concentration of calcium because leak prevented adequate alginate delivery. Abdomen did not become distended.

IAP: did not exceed 5 mm Hg during this procedure.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 112

Resuscitation fluid: warm LR (3.9 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: never started (MAP did not drop below target)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 67
- MAP: 119
- Temp: 36.9

Blood draw no. 3: (60 min post-injury): 9:39 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (60 min) VS
- HR: 62
- MAP: 117
- Temp: 34.5

Survival at 60 min? Yes

Target MAP attained? throughout

Time of death: 9:47 AM

Cause of death: intentional exsanguination from IVC transection

Interval from injury to death: 68 min

Post-treatment fluid data:
- Blood loss (intraabdominal suctioning + pads/gauze + phlebotomy):) nil, but abdomen contained 352 mL blood-tinged ascites
- IV fluid given: LR (37°C): 600 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen nondistended, with IAP = 1-2 mm Hg. No butane gas
   bubble in abdomen. Minimal foam present in lower abdomen (see Figures). Intestines in contact with foam
   was purplish/ischemic appearing, as seen before (see Figures). Blood-tinged ascites, 350 mL.
Heart: not examined.
Number of hepatic veins lacerated: NA
Portal vein injury: NA
Other: none.
Ex vivo total liver wt: liver not removed.

Tissue harvested: none

VI. COMMENTS

   Test of foam stability & toxicity for new iteration of calcium alginate foam not informative because of
failure of delivery system.
   I think it would be helpful to develop an *in vitro* system (artificial abdomen?) to work on issues on foam
delivery, firmness, & stability, before trying toxicity & injury models. That is, before utilizing more swine, we
should be reasonably confident that the delivery, firmness, & stability of the foam will not be an *in vivo* issue.

VII. PLAN

   Perhaps a group discussion would be beneficial to review short-term goals.
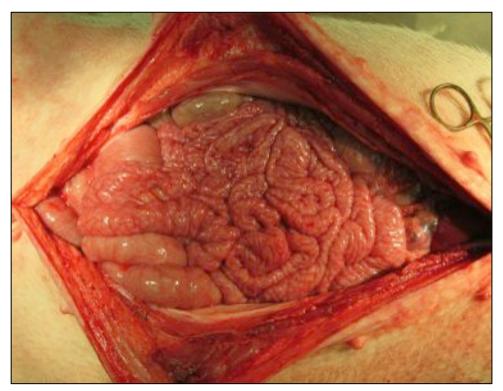
Figure 1, swine 187. Overhead view of the abdomen immediately prior to injection of calcium alginate foam,showing normal appearance of intestines. Cephalad is to the right.
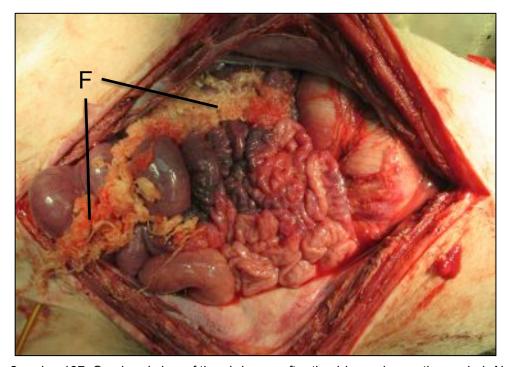
Figure 2, swine 187. Overhead view of the abdomen after the 1 hour observation period. Abdomen has been re-opened. Nothing was evacuated prior to the photo. A small amount of calcium alginate foam (F) is present. Note contrast in appearance of intestines at right (no foam contact) with those at left (+foam contact). Cephalad is to the right.

I. OVERVIEW

Date: October 29, 2013
Swine no: 188
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: Barbasol foam with all three biologics
Personnel: Carlson, Yanala, Heimann, Hansen, Noriega, Fatemi, Vanderslice, Ismail

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II. PRE-INJURY PHASE

Start time: 10:05 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 10/18/2013
Pre-procedure wt: 38.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 131
- MAP: 117
- Temp: 38.9

Blood draw no. 1 (initial): 10:14 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:22 AM
Spleen wt: 277 gm
LR (22°C) infused after splenectomy: 830 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 277 + 20 + 10 = 307 mL
- LR (22°C) infused (spleen replacement + incidental): 830 + 360 = 1190 mL

Pre-injury VS
- HR: 99
- MAP: 130
- Temp: 38.3

III. INJURY & TREATMENT PHASE

Time of injury: 10:35 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: Barbasol foam with pdFI (22.5 mg/mL), rFIIa (106 U/mL), and rFXIIIa (0.72 mg/mL).

Clotting factors: all three.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began ~30 sec after injury, after the abdomen had been completely closed with clips. After injection completed (~1 min after injury), the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Abdomen became distended during injection.

IAP: Max reading was 10, toward end of procedure.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 104

Resuscitation fluid: warm LR (3.8 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 10:36 (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 132
- MAP: 94
- Temp: 38.5

Blood draw no. 3: (60 min post-injury): 11:34 PM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (60 min) VS
- HR: 146
- MAP: 18
- Temp: 38.3

Survival at 60 min? Barely
Target MAP attained? Briefly
Time of death: 11:37 PM
Cause of death: exsanguination from liver injury
Interval from injury to death: 62 min

Post-treatment fluid data:
- Blood loss (intraabdominal suctioning + pads/gauze + phlebotomy):) 3010 + 235 + 20 mL = 3265 mL (815 g, or about 25%, of blood loss was clot)
- IV fluid given: LR (37°C): 3880 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, with IAP = 15 mm Hg. No gas pocket when incision reopened. Two clips removed and Barbasol foam, white, ~2 L,was extruded through incision with manual pressure. Most of foam removed from abdomen in this manner; most of the foam was white. All clips then removed, and incision completely re-opened. Remaining foam was mixing with unclotted blood, forming a "pink soup" that was bathing the intestines. No clotted blood was present, except in deep recesses of abdomen near the injury. There was no foam associated with the clot, which was deep dark red. Could not detect obvious evidence of intestinal toxicity, though intestines were covered with the "soup."

Heart: not examined.

Number of hepatic veins lacerated: one large vein to LL lobe (see Figs)

Portal vein injury: 2 branches, to LL lobe

Other: red clot (no foam) found in suprahepatic IVC

Ex vivo total liver wt: 896 g.

Tissue harvested: none

VI. COMMENTS

Barbasol-based foam + biologics in noncompressible model; [pdFI] in this formulation was >2x the previous formulation. No beneficial effect of biologics/foam evident. All clot appeared to be in areas not reached by foam (i.e., deep recesses); in fact, the clot and foam appeared to be mutually exclusive. That is, where there was foam, there was no clot; and where there was clot, there was no foam. That seems to have been the case with each of the Barbasol subjects. There was clot formation in this subject (~800 g), but it was all within the deepest recesses of the abdomen and appeared to be the same type of clot formed in the injury controls without treatment. I don't have any quantitative data on clot mass from previous subjects to compare. But efficacy (survival and MAP), and not clot volume, is likely the most important endpoint in this model.

After seeing the effects of Barbasol foam in the abdomen in 14 subjects now, I am quite suspicious that this carrier agent is doing something to inhibit clot formation within the porcine abdomen. I would recommend not using Barbasol foam any more.

VII. PLAN

Perhaps a group discussion would be beneficial to review short-term goals.
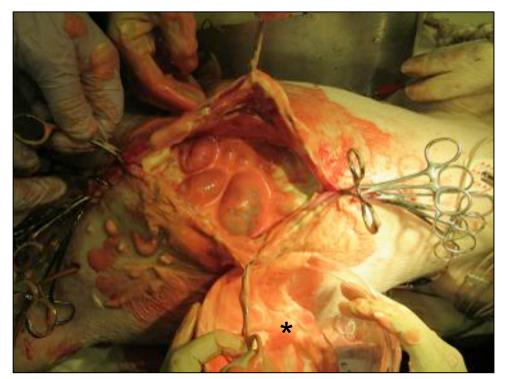
Figure 1, swine 188. Overhead view of re-opened abdomen after completion of 1 h observation; subject near expiration with a MAP of ~18. Cephalad is to the right. First ~2 L of pure white foam (no clot or any particulate) have been removed from the most anterior portion of abdomen, and now the underlying mixture of unclotted blood and foam can be seen bathing the loops of intestines in the center of the image. Beaker at bottom of image (*) is catching this "pink soup" as it leaks out of the side of the abdomen.
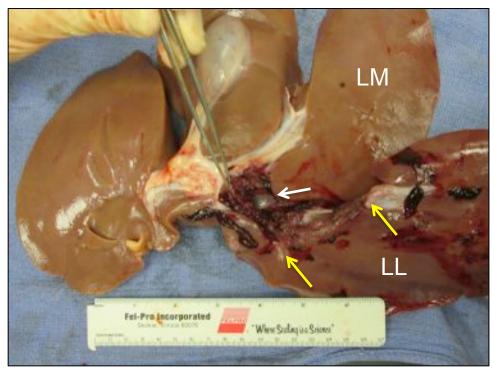
Figure 2, swine 188. Liver *ex vivo,* inferior aspect. Two branches of the PV were transected (yellow arrows). A large HV to the LL lobe was cut (white arrow), yielding a large venous injury.

I. OVERVIEW

Date: November 19, 2013
Swine no: 189
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: $CaCl_2$ and O_2 insufflation
Personnel: Carlson, Yanala, Hansen, Noriega, Fatemi

II. PRE-INJURY PHASE

Start time: 8:02 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 11/15/2013
Pre-procedure wt: 33.2 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 128
- MAP: 120
- Temp: 38.3

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:45 AM
Spleen wt: 345 gm
LR (22°C) infused after splenectomy: 1046 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 345 + 20 + 10 = 375 mL
- LR (22°C) infused (spleen replacement + incidental): 1046 + 200 = 1246 mL

Pre-injury VS
- HR: 92
- MAP: 119
- Temp: 28.7 (malfunction?)

III. INJURY & TREATMENT PHASE

Time of injury: 8:59 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. The line (IV tubing) for the oxygen was inserted through the inferior end of the midline incision and directed into the left colic gutter (see Figures).

Treatment description: 180 mL of 0.6 M $CaCl_2$, and O_2 to maintain an IAP of 10-15 mm Hg.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~2 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Insufflation of the O_2 also began 30 sec after injury. The insufflation was done using the flow regulator from a small animal anesthesia machine which was hooked to the house O_2 line without any isoflurane. Abdomen became distended during insufflation. Intermittent insufflation of O_2 was done during the observation period to maintain the IAP of 10-15.

IAP: 10-15, as above.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR (3.3 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 9:05 (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 100
- MAP: 88
- Temp: 34.9

Blood draw no. 3: (60 min post-injury): not done (omission)

Final (60 min) VS
- HR: 124
- MAP: 90
- Temp: 36.2

Survival at 60 min? Yes, easily
Target MAP attained? Intermittently throughout the observation period
Time of death: 9:05 PM

Cause of death: exsanguination from liver injury after abdomen re-opened, along with euthanasia procedure (IVC transection)
Interval from injury to death: 66 min

Post-treatment fluid data:
- Blood loss 1284 mL (suction) + 354 mL (clot) = 1638 mL
- IV fluid given: LR (37°C): 3000 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Note: subject had an episode of marked skin mottling about 10 min prior to injury. This consisted of a diffuse red skin rash, almost as if the subject was having a severe allergic reaction. Subject was hypertensive during this episode (MAP 120). Not sure what the etiology was; subject had not received any treatment other than the usual anesthetic drugs (Telazol, xylazine, ketamine, and isoflurane). Mottling spontaneously resolved, blood pressure decreased, and we continued on with procedure without any further incidents.

Findings upon abdominal/chest exploration: abdomen distended & tense with final IAP ~17 mm Hg. Upon reopening incision, large rush of gas. Moderate amount unclotted blood. Some clotted blood up around liver. Bladder & rectum very purple/discolored (see Figs), some lesser affected areas in the small intestine. Active hemorrhage as abdomen re-explored, it was difficult to determine the precise amount of blood lost prior to re-opening the inicision and that lost during abdominal exploration. I simply picked a point during the re-exploration where I thought that the fresh blood that I was seeing represented blood that was shed after I opened… this is a very rough determination.
Heart: not examined.
Number of hepatic veins lacerated: one large vein to LL lobe (see Figs)
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 925 g.

Tissue harvested: segments of dusky vs. nondusky small intestine, for H&E histology

VI. COMMENTS

Rigged system utilizing the flow regulator from the small animal anesthesia machine effective at maintaining elevated intraabdominal pressure for 1 h. No real leaks from the midline incision that was closed with towel clips. Tamponade of abdomen with O_2 insufflation to ~15 mm Hg was effective at preventing exsanguination for 1 h in our noncompressible hemorrhage model. Subject's MAP could be maintained at 90 for duration of 1 h observation period with the warm LR resuscitation. This is consistent with recent *J Trauma* paper in which tamponade with polyurethane foam was effective in a different model of noncompressible hemorrhage.

Purplish discoloration (presumably from $CaCl_2$ infusion) mostly seen in pelvic organs. This may have been a result of the calcium solution running to most dependent area in the abdomen (i.e., the pelvis)

VII. PLAN

Repeat these interventions in Swine no. 190.
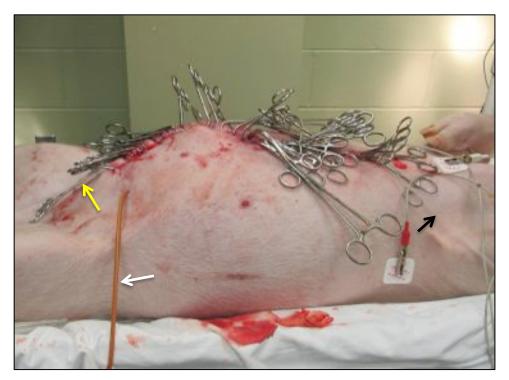
Figure 1, swine 189. Subject about 5 min after injury. Midline incision closed with towel clips. Abdomen has been insufflated with ~10 mm Hg oxygen. White arrow = transabdominal cystostomy; yellow arrow = oxygen insufflation line (entering through inferior end of incision); black arrow = intraabdominal pressure line (entering through superior end of incision). Cephalad is to the right.
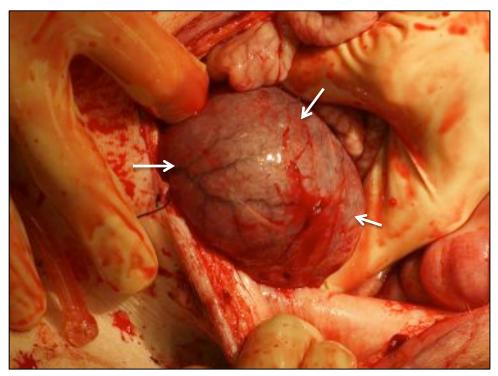
Figure 2, swine 189 necropsy. Urinary bladder (arrows) was dusky/purplish.
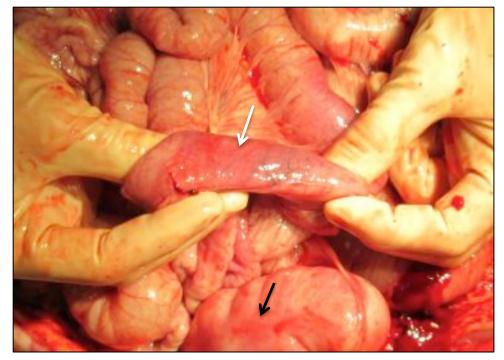
Figure 3, swine 189 necropsy. Small intestine (white arrow) and colon (black arrow) discolored. Findings much more impressive at necropsy than images can show.
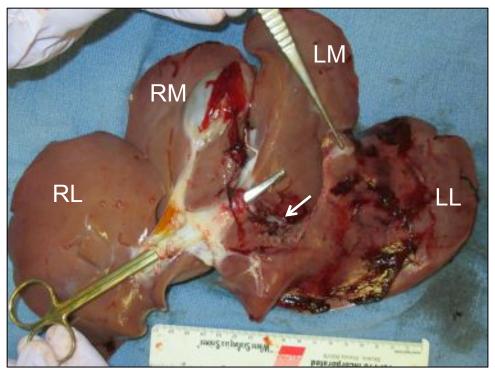
Figure 4, swine 189. Liver *ex vivo,* inferior aspect. One large branch of the PV was transected. Scissors enter through PV and exit through injury; forceps holding distal end of transected PV branch. A large HV to the LL lobe was cut (white arrow), yielding a large venous injury.

I. OVERVIEW
Date: November 19, 2013
Swine no: 190
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: $CaCl_2$ and O_2 insufflation
Personnel: Carlson, Yanala, Hansen, Noriega, Fatemi

II. PRE-INJURY PHASE

Start time: 10:30 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 11/15/2013
Pre-procedure wt: 31.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 115
- MAP: 111
- Temp: 38.8

Blood draw no. 1 (initial): 10:45 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:55 AM
Spleen wt: 364 gm
LR (22°C) infused after splenectomy: 1095 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 364 + 20 + 30 = 414 mL
- LR (22°C) infused (spleen replacement + incidental): 1095 + 200 = 1295 mL

Pre-injury VS
- HR: 98
- MAP: 132
- Temp: 38.0

III. INJURY & TREATMENT PHASE

Time of injury: 11:07 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. The line (IV tubing) for the oxygen was inserted through the inferior end of the midline incision and directed into the left colic gutter.

Treatment description: 180 mL of 0.6 M $CaCl_2$, and O_2 insufflation to maintain an IAP of 10-15 mm Hg.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~2 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Insufflation of the O_2 also began 30 sec after injury. The insufflation was done using the flow regulator from a small animal anesthesia machine which was hooked to the house O_2 line without any isoflurane. Abdomen became distended during insufflation. Intermittent insufflation of O_2 was done during the observation period to maintain the IAP of 10-15.

IAP: 10-15, as above.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 100

Resuscitation fluid: warm LR (3.1 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 11:08 (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 150
- MAP: 100
- Temp: 37.4

Blood draw no. 3: (60 min post-injury): 12:05 PM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (60 min) VS
- HR: 125
- MAP: 45
- Temp: 36.6

Survival at 60 min? Yes

Target MAP attained? Intermittently throughout the first 50 min of observation period

Time of death: 11:14 PM

Cause of death: exsanguination from liver injury after re-opening & intentional IVC transection

Interval from injury to death: 67 min

Post-treatment fluid data:
- Blood loss 1418 mL (suction) + 487 mL (clot) + 20 mL (phlebotomy) = 1925 mL
- IV fluid given: LR (37°C): 3400 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & tense with final IAP ~10 mm Hg. We insufflated to ~30 mm Hg for the final 2 min to see what would happen; no noticeable change to the vital signs. Upon reopening incision, large rush of gas. Moderate amount unclotted blood. Some clotted blood up around liver. This time the bladder & rectum were not discolored. There was some general mild discoloration of the small intestine, but otherwise a paucity of surface toxicity compared to what we have seen previously. Active hemorrhage occurred as abdomen was re-explored, it was difficult to determine the precise amount of blood lost prior to re-opening the incision and that lost subsequently during abdominal exploration. I simply picked a point during the re-exploration where I thought that the fresh blood that I was seeing represented blood that was shed after I re-opened… this was a very rough determination.
Heart: not examined.
Number of hepatic veins lacerated: one large vein to LL lobe (see Figs)
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 709 g.

Tissue harvested: segments of dusky small intestine & non-dusky stomach, for H&E histology

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VI. COMMENTS

As with no. 189, rigged system utilizing the flow regulator from the small animal anesthesia machine was effective at maintaining elevated intraabdominal pressure for 1 h. No real leaks from the midline incision that was closed with towel clips. Tamponade of abdomen with O_2 insufflation to 10-15 mm Hg was effective at preventing exsanguination for 1 h in our noncompressible hemorrhage model, though MAP was falling in final 10 min after we had run through the 100 mL/kg limit of LR resuscitation. As with no. 189, this efficacy was consistent with recent *J Trauma* paper in which tamponade with polyurethane foam was effective in a different model of noncompressible hemorrhage.

Much less purplish discoloration (presumably from $CaCl_2$ infusion) was seen in this subject. I can't really explain why subject no. 189 had more and no. 190 had less of this toxicity but, for one thing, it's really hard to make things exactly equal when one works with a highly variable surface area like the peritoneal cavity.

VII. PLAN

Repeat these procedures with Swine 191 & 192 on Tue Nov 26th.
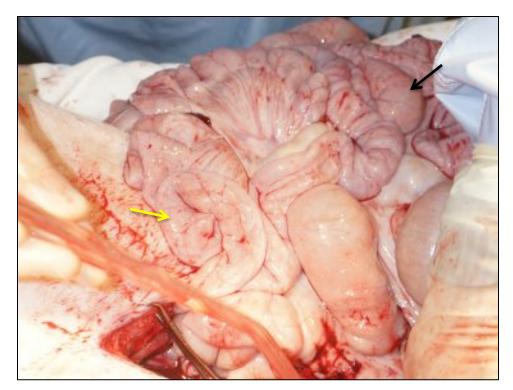
Figure 1, swine 190 necropsy. Small intestine was mildly discolored in some areas (black arrow) and less affected in other areas (yellow area). Findings not as severe as with Swine 189.
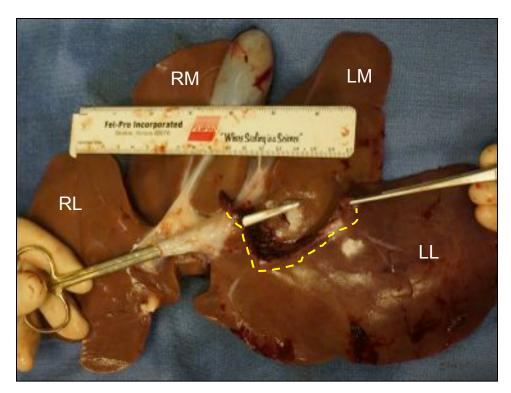
Figure 2, swine 190. Liver *ex vivo,* inferior aspect. One large branch of the PV was transected. Scissors enter through PV and exit through injury; forceps holding distal end of transected PV branch. Yellow dashed line = gap in base of LL lobe created by injury.
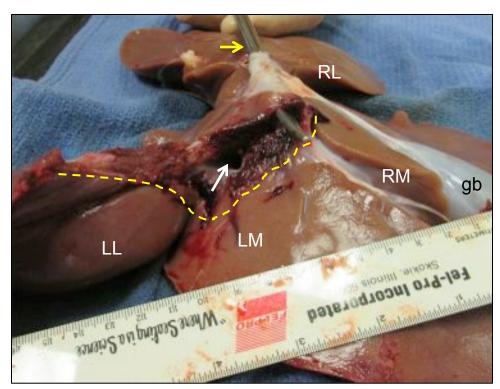
Figure 3, swine 190. Liver *ex vivo,* left oblique inferior aspect. Gaping injury in HV to LL lobe indicated by white arrow. Scissors (yellow arrow) in same position as in Fig. 2. Yellow dashed line = gap in base of LL lobe created by injury.

I. OVERVIEW
Date: November 26, 2013
Swine no: 191
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: CaCl₂ and O₂ insufflation
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi

II. PRE-INJURY PHASE

Start time: 8:04 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 11/15/2013
Pre-procedure wt: 34.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor
11. Intraabdominal O2 insufflation line

Initial VS
- HR: 112
- MAP: 106
- Temp: 38.4
- EtCO2: 41

Blood draw no. 1 (initial): 8:10 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:22 AM
Spleen wt: 254 gm
LR (22°C) infused after splenectomy: 760 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 254 + 20 + 184 = 458 mL
- LR (22°C) infused (spleen replacement + incidental): 760 + 620 = 1380 mL

Pre-injury VS
- HR: 112
- MAP: 98

- Temp: 36.7 (malfunction?)
- EtCO2: 39

III. INJURY & TREATMENT PHASE

Time of injury: 8:44 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. The line (IV tubing) for the oxygen was inserted through the inferior end of the midline incision and directed into the left colic gutter.

Treatment description: 180 mL of 0.6 M $CaCl_2$, and O_2 to maintain an IAP of 15-20 mm Hg.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~2 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Insufflation of the O_2 also began 30 sec after injury. The insufflation was done using the flow regulator from a small animal anesthesia machine which was hooked to the house O_2 line without any isoflurane. Abdomen became distended during insufflation. Intermittent insufflation of O_2 was done during the observation period to maintain the IAP of 15-20 mm Hg.

IAP: 15-20, as above.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR (3.4 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 8:45 (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 135
- MAP: 49
- Temp: 36.9
- EtCO2: 26

Blood draw no. 3: (36 min post-injury): 9:20 AM

Final (38 min) VS
- HR: 140
- MAP: 13
- Temp: 36.6
- EtCO2: 7

- IAP: 19

Survival at 60 min? No
Target MAP attained? No
Time of death: 9:23 PM
Cause of death: exsanguination from liver injury prior to re-opening abdomen
Interval from injury to death: 39 min

Post-treatment fluid data:
- Blood loss 2079 mL (suction) + 343 mL (clot) = 2422 mL
- IV fluid given: LR (37°C): 3520 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & tense with final IAP ~20 mm Hg. Upon reopening incision, large rush of gas. Large amount unclotted blood. Some clotted blood up around liver and in pelvis, but noticeably less than in previous two subjects. No obvious areas of discoloration of the intestines or other organs (see Figs). Active hemorrhage as abdomen re-explored, it was difficult to determine the precise amount of blood lost prior to re-opening the incision and that lost after incision was re-opened. I simply picked a point during the re-exploration where I thought that the fresh blood that I was seeing represented blood that was shed after I opened… this was a very rough determination.
Heart: not examined.
Number of hepatic veins lacerated: one medium-sized vein to LL lobe (see Figs)
Portal vein injury: 1 large branch, to LL lobe
Other: none
Ex vivo total liver wt: 935 g

Tissue harvested: none

VI. COMMENTS

3rd subject with noncompressible injury treated with gas insufflation and $CaCL_2$ solution only. Time course of death similar to that of untreated controls. This subject was a little unstable during the prep phase, and required extra fluid; this may have been a confounding factor. But otherwise I don't know why this one died and the previous two survived. No obvious effect of calcium solution.

VII. PLAN

Repeat these interventions in Swine no. 192.

Figure 1, swine 191. Appearance ~15 min after injury. Abdomen has been insufflated to ~20 mm Hg with O2. Midline incision closed with towel clips. 1 = O2 insufflation line; 2 = transabdominal cystotomy tube; cephalad is to the right.

Figure 2, swine 191 necropsy. Overhead view of reopened midline incision. No obvious areas of discoloration on the intestines. Cephalad is to the right.
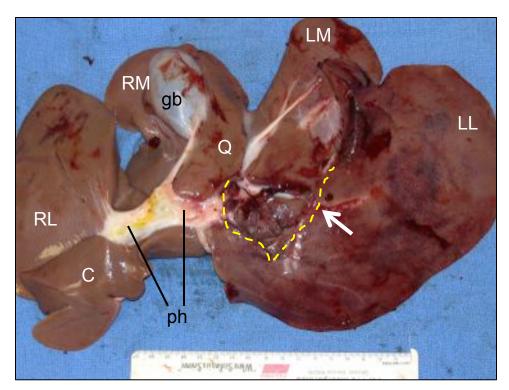
Figure 3, swine 191. Liver *ex vivo,* inferior aspect. A large branch of the PV to the LL was transected; distal end of branch indicated with arrow. Yellow dashed line = gap in base of LL lobe created by injury. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder; ph = porta hepatis.
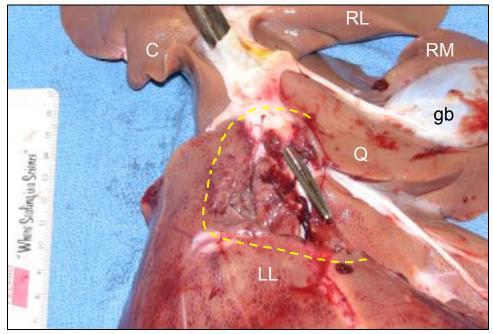
Figure 4, swine 191. Liver *ex vivo,* left oblique inferior aspect. Forceps has been inserted into the opened end of th portal vein, with the tips exiting out of the transected branch of PV to LL lobe.
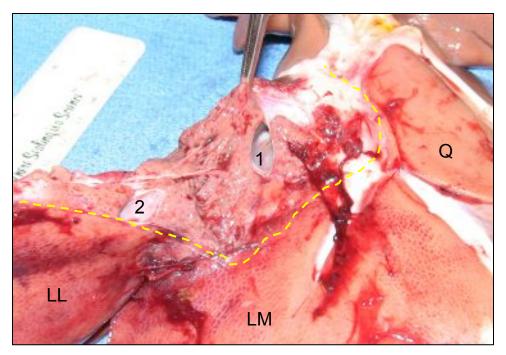
Figure 5, swine 191. Same view as in Fig. 4, but showing injury to HV to LL lobe. 1 = proximal side; 2 = distal side of HV injury.

I. OVERVIEW
Date: November 26, 2013
Swine no: 192
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: $CaCl_2$ and O_2 insufflation
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi

II. PRE-INJURY PHASE

Start time: 9:46 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 11/15/2013
Pre-procedure wt: 33.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor
11. Intraabdominal O2 insufflation line

Initial VS
- HR: 97
- MAP: 117
- Temp: 38.5
- EtCO2: 39

Blood draw no. 1 (initial): 9:58 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:10 AM
Spleen wt: 318 gm
LR (22°C) infused after splenectomy: 960 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 318 + 20 + 11 = 349 mL
- LR (22°C) infused (spleen replacement + incidental): 960 + 85 = 1045 mL

Pre-injury VS
- HR: 113
- MAP: 134

- Temp: 37.1
- EtCO2: 39

III. INJURY & TREATMENT PHASE

Time of injury: 10:21 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. The line (IV tubing) for the oxygen was inserted through the inferior end of the midline incision and directed into the left colic gutter.

Treatment description: 180 mL of 0.6 M $CaCl_2$, and O_2 insufflation to maintain an IAP of 15-20 mm Hg.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~2 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. Insufflation of the O_2 also began 30 sec after injury. The insufflation was done using the flow regulator from a small animal anesthesia machine which was hooked to the house O_2 line without any isoflurane. Abdomen became distended during insufflation. Intermittent insufflation of O_2 was done during the observation period to maintain the IAP of 15-20.

IAP: 15-20, as above.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 110

Resuscitation fluid: warm LR (3.4 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 10:23 (within 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 147
- MAP: 55
- Temp: 37.7
- EtCO2: 31

Blood draw no. 3: (49 min post-injury): 11:10 PM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Final (50 min) VS
- HR: 121
- MAP: 10
- Temp: 36.2
- EtCO2: 0

- IAP: 14

Survival at 60 min? No
Target MAP attained? No
Time of death: 11:12 PM
Cause of death: exsanguination from liver injury prior to re-opening incision
Interval from injury to death: 51 min

Post-treatment fluid data:
- Blood loss 2543 mL (suction) + 742 mL (clot) + 20 mL (phlebotomy) = 3305 mL
- IV fluid given: LR (37°C): 3620 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & tense with final IAP ~15 mm Hg. Subject
   developed rectal prolapse with extrusion of rectal temp probe. Upon reopening incision, large rush of gas.
   Large amount unclotted blood. Some clotted blood up around liver & in pelvis. No obvious discoloration of
   intestines. Active hemorrhage occurred as abdomen was re-explored, it was difficult to determine the precise
   amount of blood lost prior to re-opening the incision and that lost subsequently during abdominal exploration.
   I simply picked a point during the re-exploration where I thought that the fresh blood that I was seeing
   represented blood that was shed after I re-opened… this was a very rough determination.
Heart: not examined.
Number of hepatic veins lacerated: one hep vein to LL lobe (see Figs)
Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 787 g.

Tissue harvested: none

VI. COMMENTS

   Similar to 191, subject 192 had death from exsanguination prior to end of 1 h observation period. Not
clear to me why 189 & 190 survived easily, while 191 and 192 did not; probably ascribe this to animal model
variability. In addition, there was no obvious calcium toxicity in 191 & 192, but there was some in 189 & 190….
   I think with a high enough N (perhaps 10 in each group), I think there would be a survival advantage with
gas insufflation vs. no treatment in the noncompressible model… but I'm not sure we need to go there. Regarding
calcium toxicity, my soft conclusion is that with blood present from hemorrhage, the subjects with the
noncompressible injury probably can tolerate the small amount of free calcium leftover from the foam reaction,
but we still should strive to minimize this free calcium.

VII. PLAN

   I think we should proceed with engineering a calcium alginate formulation with improved stability. Also,
I am in the process of amending the IACUC protocol to allow 3 h observation periods, but this process will take
several months because the DoD will have to rubber stamp whatever the local IACUC approves.
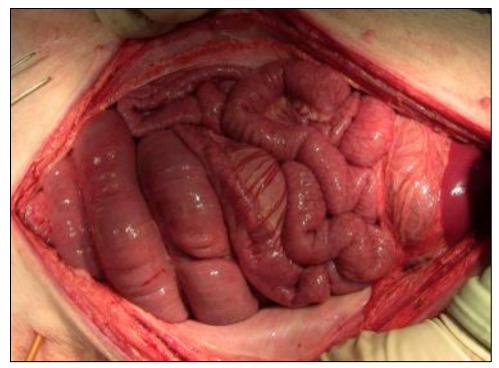
Figure 1, swine 192. Appearance of intestines prior to injury. Overhead view of midline incision, retracted open; cephalad is to the right.
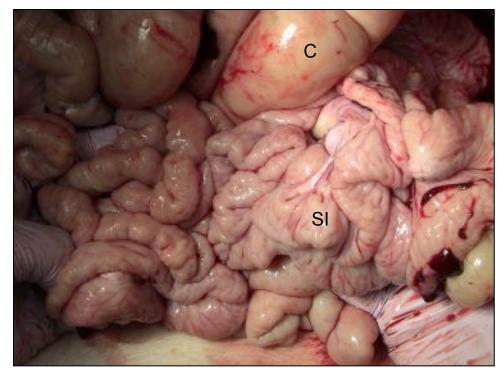
Figure 2, swine 192 necropsy. Overhead view of reopened midline incision. No obvious areas of discoloration on the intestines. Cephalad is to the right. C = colon; SI = small intestinte.
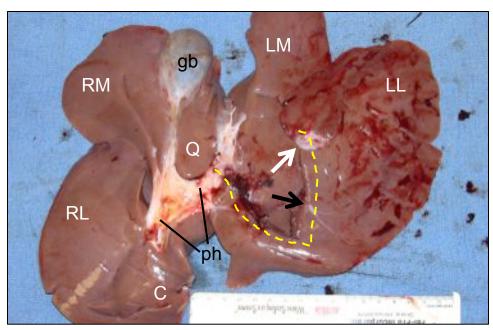
Figure 3, swine 192. Liver *ex vivo*, inferior aspect. Two branched of the PV to the LL were transected; distal end of 1st branch indicated with black arrow; distal end of 2nd branch indicated with white arrow. Yellow dashed line = gap in base of LL lobe created by injury. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder; ph = porta hepatis.
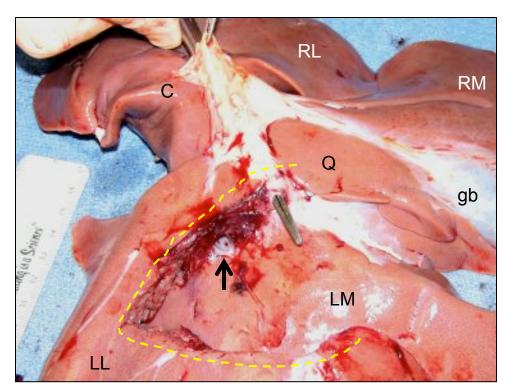
Figure 4, swine 192. Liver *ex vivo*, left oblique inferior aspect. Forceps has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Arrow indicates proximal side of transected HV to LL lobe.

I. OVERVIEW
Date: January 21, 2014
Swine no: 193
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam, new formulation
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi

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II. PRE-INJURY PHASE

Start time: 8:10 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 01/17/2014
Pre-procedure wt: 46.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 40
- MAP: 106
- Temp: 38.1
- EtCO2: 38

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:47 AM
Spleen wt: 285 gm
LR (22°C) infused after splenectomy: 850 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 285 + 20 + 29 = 334 mL
- LR (22°C) infused (spleen replacement + incidental): 850 + 180 = 1030 mL

Pre-injury VS
- HR: 82
- MAP: 133
- Temp: 37.3

- EtCO2: 31
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:59 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. *Note:* this subject had marked intestinal distension (from air/gas, etiology not clear) which was evident as soon as the abdomen was opened (Fig. 1). The intestines were difficult to pack back into the abdominal cavity after the injury.
Treatment description: calcium alginate foam, a new formulation containing higher alginate density (from 3.2% to 3.85%); no xanthan gum. Volume: 450 mL injected liquid, foams to 5.8 L, compresses to ~2 L (*in vitro* measurements per Mostafa).
Clotting factors: none.
Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began ~30 sec after injury, after the abdomen had been completely closed with clips. Shortly after injection, there appeared to a malfunction of the syringe injection pump (Harvard Apparatus), which began making strained mechanical noises, and no foam was moving through the transparent plastic lines. Only a small amount (<100 mL) actually was injected into the abdomen. The injector nozzle was withdrawn and inspected. Ultimately a plug of foam was found blocking the nozzle, which halted the flow.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 105
Resuscitation fluid: warm LR (4.6 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 9:00 (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 102
- MAP: 32
- Temp: 37.3
- EtCO2: 26

Blood draw no. 3: (60 min post-injury): 9:59 AM

Final (60 min) VS
- HR: 194
- MAP: 18
- Temp: 35.4

- EtCO2: 12
- IAP: 4

Survival at 60 min? Yes, just barely
Target MAP attained? No
Time of death: 9:01 PM
Cause of death: exsanguination from liver injury prior to re-opening abdomen
Interval from injury to death: 61 min

Post-treatment fluid data:
- Blood loss 2891 mL (suction) + 965 mL (clot) = 3856 mL
- IV fluid given: LR (37°C): 4950 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & moderately tense (IAP = 5 mm Hg). Subject essentially expired right after the 60 min observation period, before the exploration was performed. Upon re-opening abdomen, first thing visible was distending loops of bowel, but no blood (see Figs). Exploring deeper into abdomen revealed a small amount of foam mixed with clot/blood in the right gutter (see Figs), and then a large amount of clot & blood in the deeper recesses. No toxic effects to the intraabdominal organs noted.
Heart: not examined.
Number of hepatic veins lacerated: the confluence of the veins draining the LL and LM lobe was lacerated, producing a ++HV injury (see Figs).
Portal vein injury: 1 large branch, to LL lobe
Other: none
Ex vivo total liver wt: 1146 g

Tissue harvested: none

VI. COMMENTS

First trial of new iteration of Ca alginate foam. Injector tip clogged, so only a small volume of foam actually was injected. Despite the large HV injury in this subject, it survived 60 min. I would attribute this in part to the extensive intestinal distension that was present in this subject. We have not really seen this before; not sure why it was present in this subject. But the degree of distension present in this subject undoubtedly contributed to a intraabdominal tamponade effect that prolonged the subject's survival.

After the foam injector was de-clogged, the contents of the reservoir were emptied into a garbage can. After handling this foam that had been emptied into the can, I was impressed that it was stiffer than previous versions. I think that if we inject an adequate amount of this new iteration of alginate foam, then we should see treatment effect in the noncompressible model.

Of note, this subject (and the next subject) were in the 45 kg range, or about 10 kg bigger than we normally have been using. The ARDC did not have subjects in the 35 kg size range, so we took these instead. So the liver weight, resuscitation volumes, and blood loss all will be higher in these subjects.

VII. PLAN

Repeat these interventions in Swine no. 194.
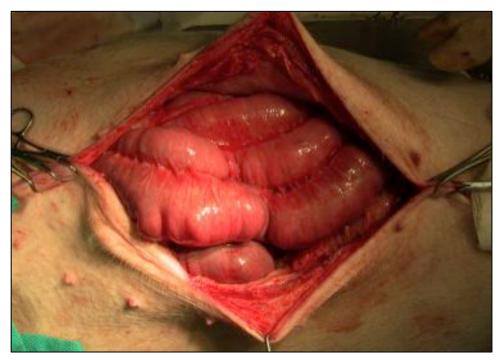
Figure 1, swine 193. Appearance of intestines prior to injury. Overhead view of midline incision, retracted open; cephalad is to the right. Intestines were distended with gas, and were difficult to keep inside the abdomen.
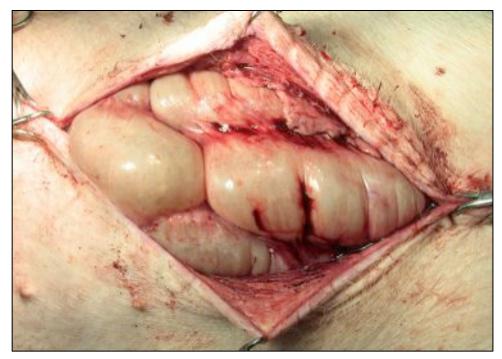
Figure 2, swine 193 necropsy. Overhead view of reopened midline incision at end of 60 min observation period. Intestines were distended with gas. No immediate evidence of hemorrhage was visible when abdomen was reopened. Cephalad is to the right.

Figure 3, swine 193 necropsy. One cluster of foam was present inside the abdomen, shown in hand. Note blood & clot mixing with foam.
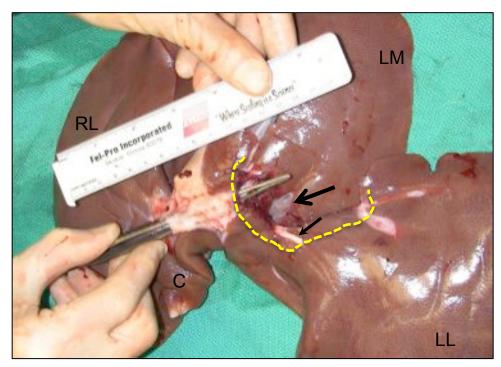

Figure 4, swine 193. Liver *ex vivo,* inferior aspect. Forceps has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Large arrow indicates transected confluence of HVs to LM & LL lobe. Small arrow indicates 2nd branch of PV to LL, not injured. RL = right lateral lobe; LM = left medial lobe; LL = left lateral lobe; dashed yellow line = separation in LL induced by cut.

I. OVERVIEW
Date: January 21, 2014
Swine no: 194
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam, new formulation
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi

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II. PRE-INJURY PHASE

Start time: 10:30 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 01/17/2014
Pre-procedure wt: 44.2 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 112
- MAP: 148
- Temp: 38.5
- EtCO2: 40

Blood draw no. 1 (initial): 11:02 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 11:16 AM
Spleen wt: 348 gm
LR (22°C) infused after splenectomy: 1050 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 348 + 20 + 107 = 475 mL
- LR (22°C) infused (spleen replacement + incidental): 1050 + 350 = 1400 mL

Pre-injury VS
- HR: 105
- MAP: 129
- Temp: 37.3

- EtCO2: 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 11:27 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: calcium alginate foam, with new formulation containing higher alginate density (from 3.2% to 3.85%); no xanthan gum. Volume: 450 mL injected liquid, foams to 5.8 L, compresses to ~2 L.

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~4 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR (4.4 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 11:28 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 178
- MAP: 27
- Temp: 36.6
- EtCO2: 12

Blood draw no. 3: (33 min post-injury): 11:51 AM

Final (34 min) VS
- HR: NA
- MAP: 10
- Temp: NA
- EtCO2: 0
- IAP: 5

Survival at 60 min? No
Target MAP attained? No

Time of death: 11:52 AM
Cause of death: exsanguination from liver injury prior to re-opening abdomen
Interval from injury to death: 34 min

Post-treatment fluid data:
- Blood loss 3805 mL (suction) + 881 mL (clot) = 4686 mL
- IV fluid given: LR (37°C): 4400 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & moderately tense with final IAP ~5 mm Hg. Upon reopening incision, moderate rush of butane gas. Some foam covering intestines superficially. Large amounts clotted & unclotted blood underneath. No obvious areas of discoloration of the intestines or other organs (see Figs). Active hemorrhage as abdomen re-explored, it was difficult to determine the precise amount of blood lost prior to re-opening the incision and that lost after incision was re-opened. As noted in no. 193, the consistency of this new iteration of foam was noticeably firmer compared to previous subjects. We measured the foam removed the abdomen at necropsy in a beaker: ~1 L.
Heart: not examined.
Number of hepatic veins lacerated: one medium-sized vein to LL lobe.
Portal vein injury: 1 large branch, to LL lobe
Other: none
Ex vivo total liver wt: 932 g
Tissue harvested: none

VI. COMMENTS

Second attempt with new iteration of the calcium alginate foam. During this procedure I continually moved the injector tip in a gentle to-and-fro fashion in hopes of avoiding a clog. We did not have a clog, but the total volume of foam recovered at necropsy (1 L) was not adequate to see a treatment effect. Subject died in less than 1 h, similar to no-treatment control.

VII. PLAN

Repeat these interventions in Swine no. 194 & 195, but with greater volume of foam, in hope of observing a treatment effect.
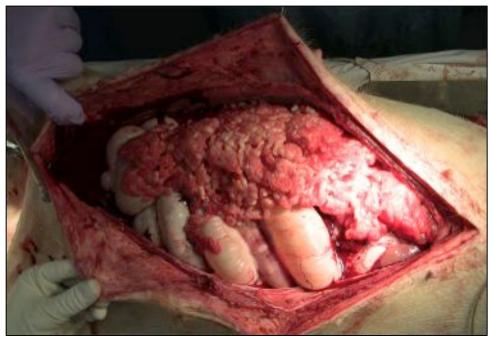
Figure 1, swine 194. Intraabdominal appearance immediately after subject death. Overhead view of midline incision, retracted open; cephalad is to the right. Moderate amount of foam covering intestines; foam volume ~1 L.
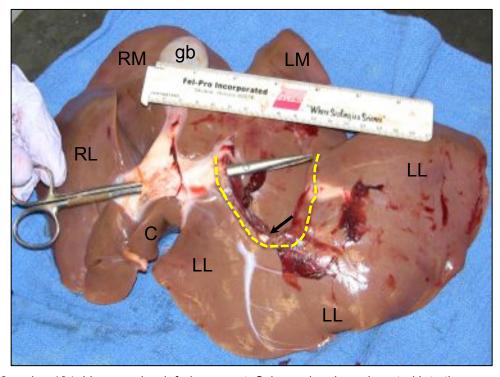
Figure 2, swine 194. Liver *ex vivo,* inferior aspect. Scissors has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Arrow indicates 2nd branch of PV to LL, not injured. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; gb = gallbladder; dashed yellow line = separation in LL induced by cut.

I. OVERVIEW
Date: January 28, 2014
Swine no: 195
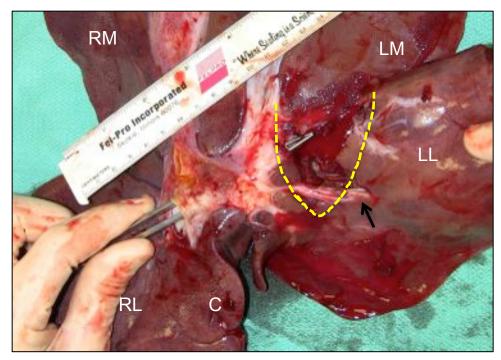Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi

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II. PRE-INJURY PHASE

Start time: 8:15 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 01/17/2014
Pre-procedure wt: 46.4 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 92
- MAP: 126
- Temp: 37.8
- EtCO2: 33

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:38 AM
Spleen wt: 366 gm
LR (22°C) infused after splenectomy: 1100 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 366 + 20 + 33 = 419 mL
- LR (22°C) infused (spleen replacement + incidental): 1100 + 300 = 1400 mL

Pre-injury VS
- HR: 98
- MAP: 107
- Temp: 37.6

- EtCO2: 30
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:49 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter. *Note:* stomach was quite distended. Left lobe of liver was small compared to previous 45 kg subjects.
Treatment description: calcium alginate foam, same formulation used in 193 & 194, containing 3.85% alginate density & no xanthan gum. Volume: 450 mL injected liquid, foams to 5.8 L, compresses to ~2 L. The gas quantity was increased from 22 to 24 g with this subject.
Clotting factors: none.
Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~4 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. *Note:* after the cut was made, I did not see the usual rush of blood, which made me wonder whether the injury was severe enough. This suspicion was enforced when we noted that the MAP did not drop as precipitously as it typically has done.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 88
Resuscitation fluid: warm LR (4.6 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 8:22 AM (within 6 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 98
- MAP: 86
- Temp: 37.1
- EtCO2: 18

Blood draw no. 3: (60 min post-injury): 9:49 AM

Final (34 min) VS
- HR: 98
- MAP: 85
- Temp: 37.0
- EtCO2: 26

- IAP: 2

Survival at 60 min? Yes
Target MAP attained? Yes, most of observation period
Time of death: 9:57 AM
Cause of death: exsanguination from euthanasia after 1 h observation period
Interval from injury to death: 68 min

Post-treatment fluid data:
- Blood loss 923 mL (suction) + 19 mL (clot) = 942 mL
- IV fluid given: LR (37°C): 3060 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not very distended, final IAP ~2 mm Hg. Upon reopening incision, some foam covering intestines superficially. Small amounts clotted & unclotted blood underneath. After foam removed, areas of purplish discoloration of the intestines were apparent (see Figs). We measured the foam removed the abdomen at necropsy in a beaker: ~0.75 L.
Heart: not examined.
Number of hepatic veins lacerated: one medium-sized vein to LL lobe.
Portal vein injury: 1 medium branch, to LL lobe
Other: none
Ex vivo total liver wt: 1195 g (the left lateral lobe was noticeably smaller compared to previous 45 kg subjects)
Tissue harvested: none

VI. COMMENTS

This animal survived the 1 h observation period easily, but bleeding was not at expected level even though the injury was adequate. Not sure why this was… the LL lobe was smaller than expected, perhaps this was the issue. The stomach also was quite distended, perhaps this produced local mechanical changes that stemmed the bleeding. But there was not an adequate volume of foam injected to have made a treatment effect. We still need to get a higher foam volume injected (~5 L), with 2-3 L recovery after 1 h.

VII. PLAN

Repeat these interventions in Swine no. 196, but with greater gas quantity.
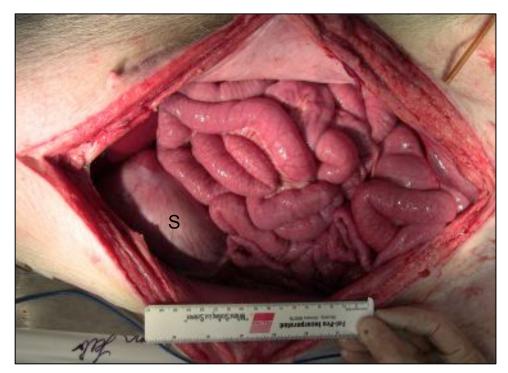
Figure 1, swine 195. Appearance of intestines prior to injury. Overhead view of midline incision, retracted open; cephalad is to the left. Stomach (S) was distended.
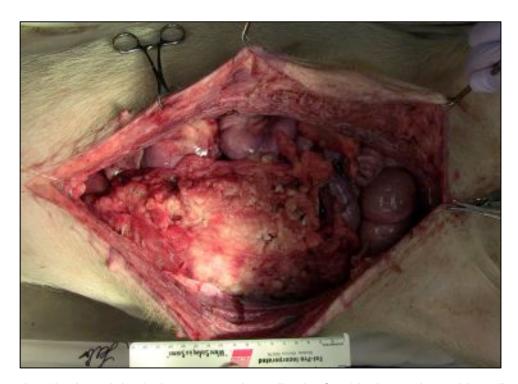
Figure 2, swine 195. Intraabdominal appearance immediately after 1 h observation; subject alive & well. Overhead view of midline incision, retracted open; cephalad is to the left. Moderate amount of foam covering intestines; foam volume ~0.75 L.
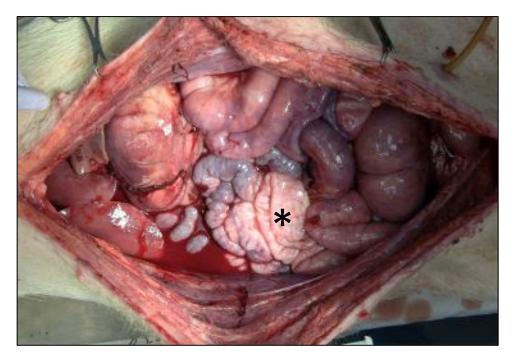
Figure 3, swine 195. Intraabdominal appearance after 1 h observation with foam removed; subject still alive, but BP dropping. Overhead view of midline incision, retracted open; cephalad is to the left. Compared to the typical subject, there was decreased blood in the abdomen in no. 195. Note the ischemic appearance of the bowel loops (unaffected region shown with asterisk).
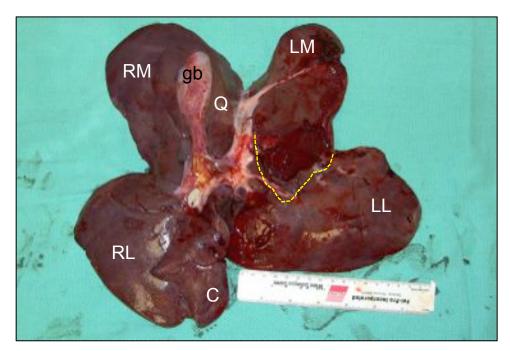
Figure 4, swine 195. Liver *ex vivo,* inferior aspect. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder; dashed yellow line = separation in LL induced by cut.
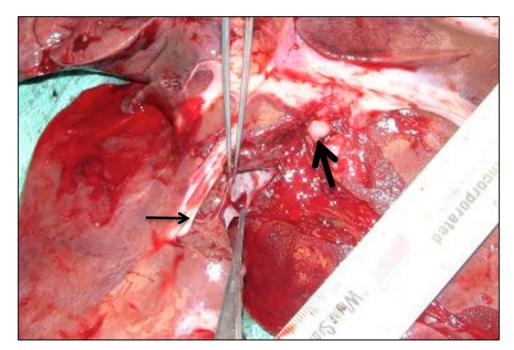
Figure 5, swine 195. Liver *ex vivo*, inferior aspect, oblique view. Forceps are demonstrating injury to HV draining the LL lobe. Thin arrow: 2nd branch of PV to LL lobe (not injured). Thick arrow: transected proximal end of 1st branch of PV to LL lobe.

Figure 6, swine 195. Liver *ex vivo,* inferior aspect. Forceps has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Arrow indicates indicates 2nd branch of PV to LL, not injured. Dashed yellow line = separation in LL induced by cut.

I. OVERVIEW
Date: January 28, 2014
Swine no: 196
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi

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II. PRE-INJURY PHASE

Start time: 10:18 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 01/17/2014
Pre-procedure wt: 48.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 84
- MAP: 131
- Temp: 38.4
- EtCO2: 35

Blood draw no. 1 (initial): 10:26 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:44 AM
Spleen wt: 310 gm
LR (22°C) infused after splenectomy: 930 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 310 + 20 + 38 = 368 mL
- LR (22°C) infused (spleen replacement + incidental): 930 + 250 = 1180 mL

Pre-injury VS
- HR: 94
- MAP: 142
- Temp: 37.0

- EtCO2: 18
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 10:53 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: calcium alginate foam, same formulation used in 193 & 194 (except gas, see below), containing 3.85% alginate density & no xanthan gum. Volume: 450 mL injected liquid, foams to 5.8 L, compresses to ~2 L. The gas quantity was increased to 25 g with this subject (24 g used with no. 195).

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~4 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 115

Resuscitation fluid: warm LR (4.9 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 10:54 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 90
- MAP: 49
- Temp: 37.5
- EtCO2: na

Blood draw no. 3: (60 min post-injury): 11:53 AM

Final (60 min) VS
- HR: 67
- MAP: 30
- Temp: 37.5
- EtCO2: 18
- IAP: 5

Survival at 60 min? Yes

Target MAP attained? Intermittently, early on
Time of death: 12:00 PM
Cause of death: exsanguination from euthanasia after 1 h observation period
Interval from injury to death: 67 min

Post-treatment fluid data:
- Blood loss 2773 mL (suction) + 771 mL (clot) = 3544 mL
- IV fluid given: LR (37°C): 5060 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, final IAP ~5 mm Hg. Upon reopening incision, some foam covering intestines superficially, mostly in right gutter. Large amounts clotted & unclotted blood underneath, partially mixed with foam. After foam removed, areas of purplish discoloration of stomach & intestines were apparent (see Figs). We measured the foam removed the abdomen at necropsy in a beaker: ~0.75 L.
Heart: not examined.
Number of hepatic veins lacerated: one medium-sized vein to LL lobe.
Portal vein injury: 1 medium branch, to LL lobe
Other: none
Ex vivo total liver wt: 1195 g
Tissue harvested: none

VI. COMMENTS

This animal barely survived the 1 h observation period, but probably would have died within the next 15 min. Subjectively, more foam seemed to have been injected in this subject than in no. 195, but still not really enough to see a treatment effect. One encouraging observation with the alginate foam is that it mixes fairly well with the blood; clot forms in and around the alginate foam, which we did not see with the Barbasol foam (the latter appeared to inhibit clotting).

One concerning observation we saw in no. 195 & 196, however, was the return of purplish/ischemic intestine. This discoloration appeared to be where the bowel was in contact with the foam (i.e., there were obvious lines of demarcation between discolored bowel and normal). We will have to be vigilant about this side effect, because it could torpedo our efforts if/when we arrive the point where we want to obtain permission to use this device in humans.

VII. PLAN

I would like to repeat this interventions again in the next two subjects, with a focus on delivering at least 5 L of foam initially and retrieving 2-3 L of foam after the 1 h observation period. I will be out of town the week of Feb 3rd, so the next swine procedure date will be Tue Feb 11th.
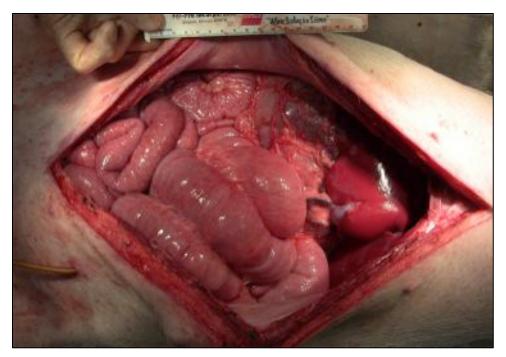
Figure 1, swine 196. Appearance of intestines prior to injury. Overhead view of midline incision, retracted open; cephalad is to the right.

Figure 2, swine 196. Intraabdominal appearance immediately after 1 h observation; subject alive, MAP low (~30). Overhead view of midline incision, retracted open; cephalad is to the right. Moderate amount of foam in right gutter; foam volume ~0.75 L.
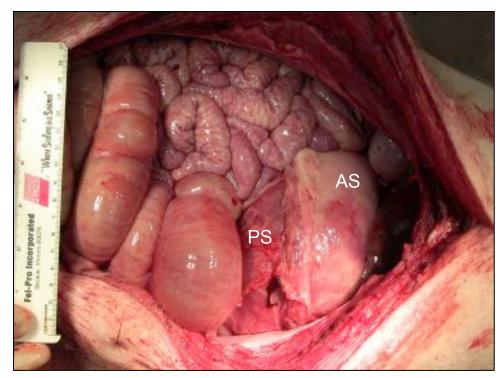
Figure 3, swine 196. Intraabdominal appearance after 1 h observation with foam removed; subject still with measurable MAP (~25). Overhead view of midline incision, retracted open; cephalad is to the right. Loops of bowel diffusely ischemic appearing. Note contrast in color of the posterior stomach (PS; in contact with foam) compared to the anterior stomach (AS; not in contact with foam).
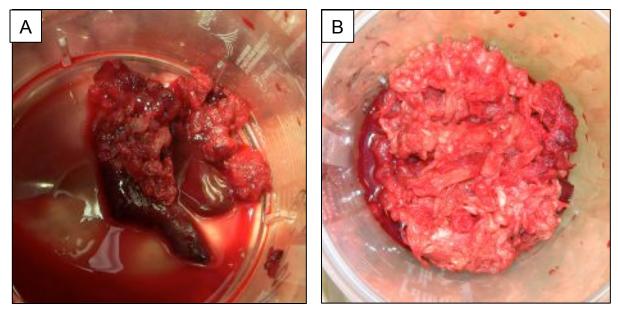
Figure 4, swine 196. Images of foam removed after 1 h observation period. (A) Small sample of foam mixed with clotted blood, shown at bottom of a 2 L plastic beaker. (B) Total foam removed from the abdomen after 1 h, volume ~750 mL.
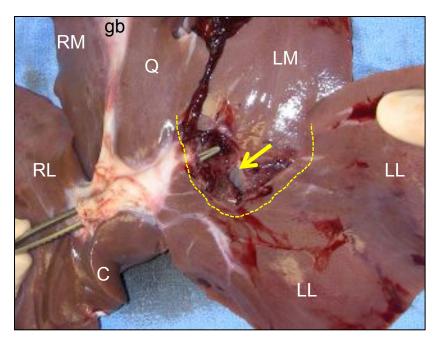
Figure 5, swine 196. Liver *ex vivo,* inferior aspect. Forceps has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Arrow indicates lumen of transected hepatic vein to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.
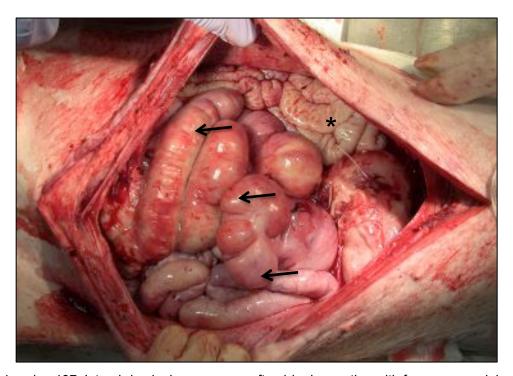
I. OVERVIEW

Date: February 11, 2014
Swine no: 197
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi, Noriega

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II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/07/2014
Pre-procedure wt: 35.4 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
   1. Endotracheal tube with ETCO2 monitor
   2. EKG clips
   3. Left ear vein angiocath (20g) for supplemental LR
   4. Right carotid artery angiocath (20g), cutdown; for BP monitor
   5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
   6. Transabdominal cystotomy for 16 Fr Foley catheter
   7. Rectal temp probe
   8. Pulse oximetry
   9. Heating pad below subject
   10. Intraabdominal pressure monitor

Initial VS
   • HR: 87
   • MAP: 109
   • Temp: 37.2
   • EtCO2: 34

Blood draw no. 1 (initial): 08:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:26 AM
Spleen wt: 250 gm
LR (22°C) infused after splenectomy: 750 mL at 150 mL/min

Pre-injury fluid data:
   • Blood loss (spleen weight + phlebotomies + incidental): 250 + 20 + 66 = 336 mL
   • LR (22°C) infused (spleen replacement + incidental): 750 + 175 = 925 mL

Pre-injury VS
   • HR: 128
   • MAP: 106
   • Temp: 35.6

- EtCO2: 31
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:41 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium alginate injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.
Treatment description: calcium alginate foam, same formulation used in 195 & 196 (3.8% alginate, 0.6% Tween 20 in DI H_2O; volume = 450 mL injected liquid, which foams to 5.8 L, compresses to ~2 L *in vitro*). The butane gas quantity was increased to 25 g with this subject (24 g used with no. 196). About 170 mL out of 450 mL of the formulation actually was injected into the subject.
Clotting factors: none.
Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~4 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 85
Resuscitation fluid: warm LR (3.5 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 08:42 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 145
- MAP: 52
- Temp: 35.9
- EtCO2: 24
- IAP: 19

Blood draw no. 3: (60 min post-injury): 09:40 AM

Final (60 min) VS
- HR: 154
- MAP: 34
- Temp: 35.2
- EtCO2: 18
- IAP: 8

Survival at 60 min? Yes
Target MAP attained? Briefly, early on
Time of death: 9:47 PM
Cause of death: exsanguination from euthanasia after 1 h observation period
Interval from injury to death: 66 min

Post-treatment fluid data:
- Blood loss 2555 mL (suction) + 267 mL (clot) = 2822 mL
- IV fluid given: LR (37°C): 3620 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, final IAP ~8 mm Hg. Upon reopening incision, foam was covering intestines superficially, and bulged a little bit out of the incision as it was reopened (see Figs). Large amounts clotted & unclotted blood underneath, partially mixed with foam. After foam removed, some mild ischemic changes of intestine noted (see Figs). Foam volume recovered at necropsy in a beaker: ~0.75 L.
Heart: not examined.
Number of hepatic veins lacerated: one medium-sized vein to LL lobe.
Portal vein injury: 2 medium branches, to LL lobe
Other: none
Ex vivo total liver wt: 849 g
Tissue harvested: none

VI. COMMENTS

This animal survived the 1 h observation period, but probably would have expired during the next 30-45 min. Qualitatively, the foam appears to have functioned better in this subject, as it bulged through the incision at the reopening for necropsy. We also had a higher IAP than we have been seeing recently—up to 19 mm Hg at one point, then settling back to 8. But the recovered foam volume was still too low; of note, only 170 of the 450 mL formulation (38%) actually was dispensed into the abdomen. The foam consistency and firmness seem appropriate, we just need to be able to dispense more foam so that we can obtain a higher 60 min foam recovery volume.

Only minor ischemic changes to the intestines noted in this subject.

VII. PLAN

Increase butane gas quantity in the next subject to increase the fraction of the formulation that is dispensed into the abdomen.

Figure 1, swine 197. Appearance of foam as it was injected out of canister at the end of the injection sequence. Foam was firm, like a little sausage.

Figure 2, swine 197. Intraabdominal appearance immediately after 1 h observation; subject alive, MAP in the 30's. Overhead view of midline incision, retracted open; cephalad is to the right. Foam was located anteriorly, and bulged from the abdomen when the incision was re-opened.; foam volume was ~0.75 L.
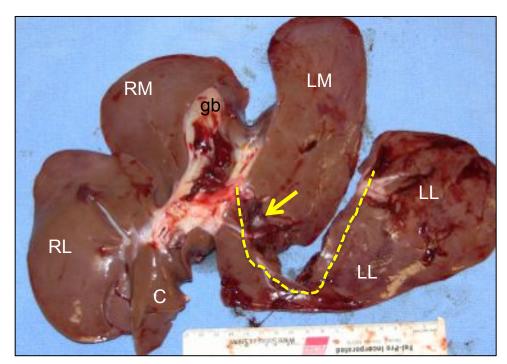
Figure 3, swine 197. Liver *ex vivo,* inferior aspect. Arrow indicates lumen of transected hepatic vein to LL lobe. Dashed yellow line = gap in LL lobe induced by cut (this injury was a near-complete transection of the LLL). RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; gb = gallbladder.

Figure 4, swine 197. Intraabdominal appearance after 1 h observation with foam removed; image taken after euthanasia. Overhead view of midline incision, retracted open; cephalad is to the right. Loops of bowel with some mild ischemia in the anterior position (arrows); loops located in right upper quadrant (*) appear normal.

I. OVERVIEW
Date: February 11, 2014
Swine no: 198
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi, Noriega

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II. PRE-INJURY PHASE

Start time: 10:00 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/07/2014
Pre-procedure wt: 34.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 122
- MAP: 84
- Temp: 39.1
- EtCO2: 39

Blood draw no. 1 (initial): 10:16 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:30 AM
Spleen wt: 271 gm
LR (22°C) infused after splenectomy: 815 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 310 + 20 + 38 = 368 mL
- LR (22°C) infused (spleen replacement + incidental): 815 + 140 = 955 mL

Pre-injury VS
- HR: 119
- MAP: 107
- Temp: 37.3

- EtCO2: 31
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 10:46 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium alginate injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: same formulation as 197, but with 90 g butane (25 g used in no. 197). Vol dispensed into abdomen = 360 mL out of the possible 450 mL (80%).

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~4 min to complete. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. *Note*: injection was much stronger & quicker with the increased butane. The subject's abdomen rapidly distended, with the IAP immediately going above 20. We had some leaks of gas and foam through the clips that we had to fix. Overall, injection took less than half the time of previous injections.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 85

Resuscitation fluid: warm LR (3.5 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 10:47 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 145
- MAP: 40
- Temp: 37.5
- EtCO2: 22
- IAP: 24

Blood draw no. 3: (59 min post-injury): 11:45 AM

Final (58 min) VS
- HR: 40
- MAP: 8
- Temp: 37.0
- EtCO2: 5

- IAP: 15

Survival at 60 min? No
Target MAP attained? No
Time of death: 11:44 AM
Cause of death: exsanguination from injury
Interval from injury to death: 58 min

Post-treatment fluid data:
- Blood loss 2891 mL (suction) + 546 mL (clot) = 3437 mL
- IV fluid given: LR (37°C): 3740 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, with final IAP ~15 mm Hg. There was distension/dissection into the right medial thigh (see Figures). Upon reopening incision, foam was covering intestines superficially, and bulged through the incision as it was reopened (see Figs). Large amounts clotted & unclotted blood underneath, partially mixed with foam. After foam removed, some mild ischemic changes of intestine noted (see Figs). Foam volume recovered at necropsy in a beaker: ~1.1 L.
Heart: not examined.
Number of hepatic veins lacerated: large injury = confluence of veins to LL and LM lobes (see Figs).
Portal vein injury: 2 medium branches, to LL lobe
Other: none
Ex vivo total liver wt: 835 g
Tissue harvested: none

VI. COMMENTS

Technical result with this subject was improved, though subject did not survive the hour. The injury to the hepatic veins in this subject was particularly severe. The increased quantity of butane used in this subject made for a vigorous foam injection, and increased the fraction of the alginate formulation that was dispensed into the abdomen. Part of the tamponade effect we saw today probably was from the gas bubble inside the abdomen; this was present to a marked degree early on, but the gas slowly leaked out of the belly as the hour progressed. I still think we need to have a one-hr foam recovery of >2 L, though… but we did recover 1.1 L of foam today, which was better than previously seen. So some progress…

Similar to no. 197, only mild ischemic changes seen on the intestines of this subject at necropsy.

VII. PLAN

Next two subjects will be operated in one week: Tue Feb 18th. We will continue trialing/improving the foam device so that we can obtain a >2 L foam recovery at one hour.

There are two areas of data which I think would be helpful to this project: (1) some quantification of foam firmness, so that we can report this quantity in a future manuscript, instead of giving a qualitative description; and (2) assay of the cation concentrations (calcium, sodium) which are present in the small aqueous portion of the foam that is present after an *in vitro* test injection. I discussed such possibilities with the team today.
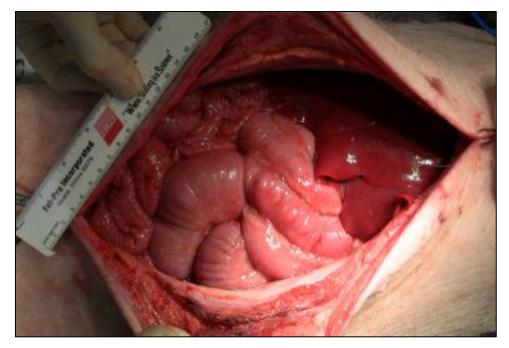
Figure 1, swine 198. Appearance of intraadominal viscera prior to injury. Cephalad is to the right.
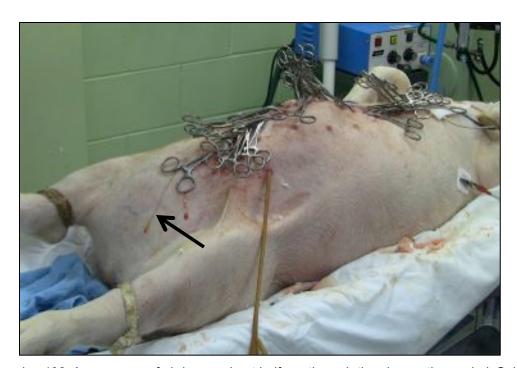
Figure 2, swine 198. Appearance of abdomen about halfway through the observation period. Subject alive with MAP in the 30's. Note severe distension with dissection into the right medial thigh (arrow).

Figure 3, swine 198. Intraabdominal appearance about 62 min after injury; subject expired at 58 min. Overhead view of midline incision, retracted open; cephalad is to the right. Foam was located anteriorly, and bulged from the abdomen when the incision was re-opened.; foam volume was ~1.1 L. Inset: foam ex vivo, showing maintenance of shape.
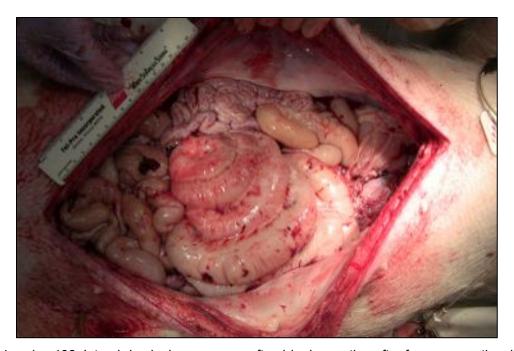
Figure 4, swine 198. Intraabdominal appearance after 1 h observation after foam evacuation; liver has already been removed. Overhead view of midline incision, retracted open; cephalad is to the right. Only a modest sugestion of ischemia was present.
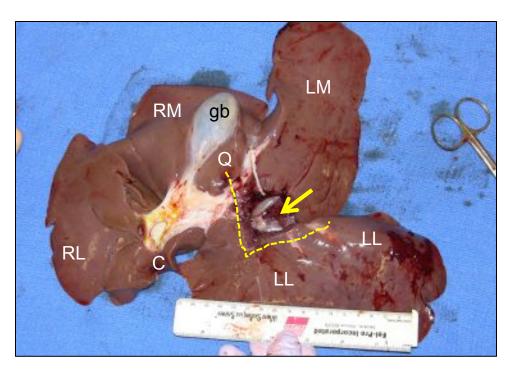
Figure 5, swine 198. Liver *ex vivo,* inferior aspect. Arrow indicates lumen of transected confluence of hepatic veins to LM & LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrant lobe; C = caudate lobe; gb = gallbladder.
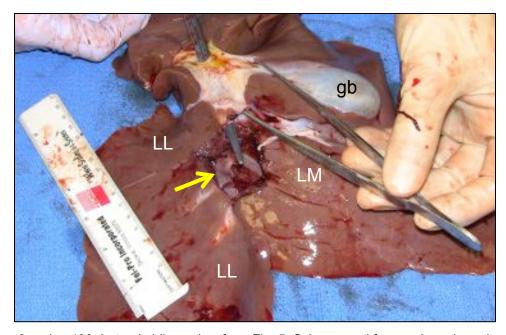
Figure 6, swine 198. Lateral oblique view from Fig. 5. Scissors and forceps have been inserted into lumen of two transected branches of PV to LLL. Arrow indicates lumen of transected confluence of hepatic veins to LM & LL lobe.

I. OVERVIEW
Date: February 25, 2014
Swine no: 199
Model: swine, normothermic, normovolemic noncompressible hemorrhage; **no injury control**
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Cavanaugh, Hansen, Heimann, Fatemi, Noriega

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II. PRE-INJURY PHASE

Start time: 8:10 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/07/2014
Pre-procedure wt: 37.8 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 118
- MAP: 122
- Temp: 38.6
- EtCO2: 15

Blood draw no. 1 (initial): 08:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:51 AM
Spleen wt: 329 gm
LR (22°C) infused after splenectomy: 990 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 329 + 20 + 40 = 389 mL
- LR (22°C) infused (spleen replacement + incidental): 990 + 160 = 1150 mL

Pre-injury VS
- HR: 134
- MAP: 122
- Temp: 36.8

- EtCO2: 15
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of 1st foam injection: 09:04 AM

Injury type: **none**; today was foam injection only. Prior to the injection, the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium alginate injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment description: similar (not same) formulation as subject no. 198, specifically: 120 g butane, 3.8% alginate, 0.6% Tween 20 in DI H_2O; precursor volume = 800 mL (almost double the volume from previous subjects); $CaCl_2$ = 1.14 M; calcium pump flow rate = 28 mL/min.

Clotting factors: none.

Technique: The calcium alginate injection was performed for a little over 1 min, which was the time it required to empty the canister during *in vitro* testing. The excess butane gas was bled out of the abdomen by temporarily releasing several clips. After injection, the abdomen was only mildly distended, and the IAP only rose several mm Hg. The canister was checked, and it became apparent that only a fraction of the total volume had been injected (~680 mL or 85% was still in the canister, so only 15% of the canister contents were injected). The abdomen was re-opened, and the injected foam was removed for measurement: ~1.5 L (see Figures). At this point is was not possible to continue injection with the 1st canister, so we decided to prepare a 2nd canister and perform a secondary foam injection on the same subject, the VS of which were still stable.

VS prior to 2nd foam injection: HR 130; MAP 95; T 35.9, EtCO2 15

Time of 2nd foam injection: 09:50 AM

Parameter were similar to above, except that the calcium flow rate was 21 mL/min, and the injection was carried out for 3+ min, until the calcium syringes were emptied. The liquid volume injected this time was 575 mL, which was ~70% of the canister contents. During the injection, the intraabdominal pressure rose transiently to ~20 mm Hg, and the abdomen was very tight and distended. Some dissection of tissues (subcutaneous emphysema) occurred in the groin areas. The excess butane gas was bled out of the abdomen again by temporarily releasing several clips. After the gas was bled out, the IAP dropped backdown to ~12 mm Hg. The subjects BP increased 20-30 mm Hg during this injection (he was somewhat light under the anesthetic), but dropped back down after the gas was bled out.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 76

Resuscitation fluid: warm LR (3.8 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: very little administered

IV. POST-TREATMENT PHASE

Blood draw no. 2 (56 min post-injury): 10:46 AM

Final (60 min) VS
- HR: 99
- MAP: 76
- Temp: 35.1
- EtCO2: 18
- IAP: 8

Survival at 60 min? Yes
Target MAP attained? Yes, throughout
Time of death: 10:58 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 58 min

Post-treatment fluid data:
- Blood loss; minimal
- IV fluid given: LR (37°C): <1 L

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, final IAP had drifted down to 8 mm Hg. There
   was distension/dissection into the right medial thigh. Upon reopening incision, foam was covering intestines,
   and bulged through the incision as it was reopened (see Figs). Foam mass was removed as one intact
   specimen (see Figures). After foam removed, ischemic changes of intestine noted (see Figs). There was
   several hundred mL of red-tinged ascites in the peritoneal cavity. Foam volume recovered at necropsy in a
   beaker: 2+ L.
Heart: not examined.
Number of hepatic veins lacerated: NA
Portal vein injury: NA
Other: none
Ex vivo total liver wt: NA g
Tissue harvested: none

VI. COMMENTS

   No-injury control subject today, with the intent to measure foam volume recovery. Larger canister used
today carrying 800 mL of the liquid alginate component. The injection time required to empty the canister *in vitro*
does not correlate with the *in vivo* time, that is, the injection goes much quicker when performed on the bench
than in the animal. This bench-animal inconsistency was why the volume injection with the 1st attempt was not
adequate. With the 2nd injection, an increased foam volume was recovered, a little over 2 L. But this is still not
enough; I would predict that we will need to recover 3-4 L to see a treatment effect when an injury is performed.
But 2+ L is still better than we have seen before, so we are improving.
   At this phase we still need to bleed of the excess butane during the injection procedure, otherwise the
abdominal distension becomes excessive. And tamponade from any gas injection typically is lost during the
ensuring observation period… that is, the tamponade effect needs to come from the space-occupying lesion that is
the calcium alginate foam, but not from the gas.
   This subject had marked changes to the visceral surfaces, but that probably was secondary to the fact that
(i) there was no blood to dilute the free calcium, and (ii) two sequential injections were performed.

VII. PLAN

   Repeat this protocol in next subject on Tue March 4th. For control no-injury subjects, I think it is
reasonable to get two injections out of one subject, so we will only need to utilize one subject on 3/4/14.

Fig. 1, swine 199. Volume of foam (~1.5 L) recovered from the abdomen immediately after the 1st injection.

Fig. 2, swine 199. Appearance of abdomen at reopening 1 h after the 2nd foam injection. Cephalad is to the right (intraabdominal pressure monitor exiting at op of incision).

A

Fig. 3, swine 199. (A) Foam mass from Fig. 2 lifted out of the abdominal cavity as one intact specimen. (B) Volume of foam mass: 2+ L.

B
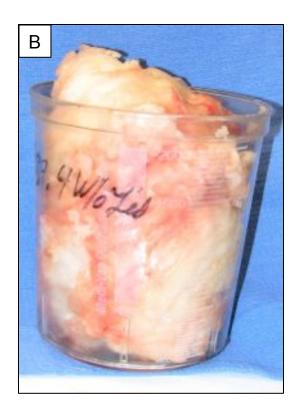
Figure 4, swine 199. Intraabdominal appearance after 1 h observation and foam evacuation. Overhead view of midline incision, retracted open; cephalad is to the right. Visceral surfaces were diffusely purplish and ischemic-looking.

I. OVERVIEW
Date: March 6, 2014
Swine no: 200
Model: swine, normothermic, normovolemic noncompressible hemorrhage; **no injury control**
Treatment: calcium alginate foam
Personnel: Carlson, Hansen, Heimann, Fatemi, Noriega

II. PRE-INJURY PHASE

Start time: 9:15 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/07/2014
Pre-procedure wt: 38.4 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 129
- MAP: 91
- Temp: 38.4
- EtCO2: 30

Blood draw no. 1 (initial): 09:27 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:45 AM
Spleen wt: 310 gm
LR (22°C) infused after splenectomy: 930 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 310 + 20 + 10 = 340 mL
- LR (22°C) infused (spleen replacement + incidental): 930 + 130 = 1090 mL

Pre-injury VS
- HR: 107
- MAP: 98
- Temp: 36.3

- EtCO2: 29
- IAP: 0

III. INJURY & TREATMENT PHASE

Initiation of 1st series of foam injections: 10:03 AM
Injury type: **none**; today was foam injection only. Prior to the injection, the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the calcium alginate injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.
Treatment description: similar (not same) formulation as subject no. 198, specifically: 120 g butane, 3.8% alginate, 0.6% Tween 20 in DI H_20; precursor volume = 800 mL (almost double the volume from previous subjects); $CaCl_2$ = 1.14 M; calcium pump flow rate = 21 mL/min/syringe x 4 syringe = 84 mL/min (see Figs).
Clotting factors: none.
Observations: The initial calcium alginate injection was carried out over ~4 min, during which butane gas was continually bled out of the incision by releasing towel clips and also by having a plastic venting tube within the abdomen and exiting through the superior portion of the incision. The IAP rose temporarily to over 20 mm Hg after the initial injection, but then steadily declined to <10 during the next several min. Therefore, a secondary injection of calcium alginate using a new container of alginate was performed about 10 min into the procedure. During this secondary injection the butane gas also was vented, but the IAP peaked at ~45 mm Hg (see Figs), and the injection was stopped. A smaller volume of foam was instilled during the secondary injection; there simply was not any more room in the peritoneal cavity for more foam. VS did not change much during this injection, even with a very high IAP. The IAP gradually declined over the rest of the one hour observation period, and was <5 at hour's end, but no obvious leaks were present in the incision. When the abdomen was re-opened, there were no pockets of butane.
A large mass of foam was overlying the intestines (see Figs), and was >2 L in volume. *Note:* the foam was only present anteriorly overlying the intestines, but no where else. That is, the foam did not extend into any far corner of the abdomen, inferior to the liver, down into the pelvis, etc. The foam only was present in the immediate region of injection, that is, the potential space that exits anterior to the viscera and posterior to the abdominal wall/incision.
After foam evacuation, the visceral surfaces were noted to have purplish discoloration (see Figs), and there was was about 100 mL of pinkish red ascites.
Mass of alginate + butane injected = 885 g

VS prior to 2nd series of foam injection: HR 140; MAP 81; T 34.7, EtCO2 22
Initiation of 1st series of foam injections: 11:36 AM
Injury type: see above
Treatment description: see above
Clotting factors: none
Observations: similar to above. A primary injection was performed, followed about 15 min later (after the IAP had decreased to <10) with a secondary injection. The IAP increased to the mid-20's after the secondary injection, and remained in the teens until the 2nd one-hour observation period was over. When the abdomen was re-opened, there was a small pocket of butane just under the incision. A large mass of foam was overlying the intestines (see Figs), and was >2 L in volume (see above note). The visceral surfaces had purplish discoloration, and there was several hundred mL of pinkish red ascites.
Mass of alginate + butane injected = 1149 g

IV. POST-TREATMENT PHASE

Blood draw no. 2: NA

Final VS after completion of 2nd one-hour observation period
- HR: 85
- MAP: 79
- Temp: 33.4
- EtCO2: 18
- IAP: 14

Survival at end: Yes
Target MAP attained? Yes, throughout, with some fluid resuscitation
Time of death: 12:30 PM
Cause of death: exsanguination from euthanasia
Interval from initiation to death: 165 min

Post-treatment fluid data:
- Blood loss; minimal (no injuries done)
- IV fluid given: LR (37°C): ~2 L

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings as above.
Heart: not examined.
Number of hepatic veins lacerated: NA
Portal vein injury: NA
Other: none
Ex vivo total liver wt: NA g
Tissue harvested & fixed in formalin: (1) ischemic-appearing small intestine; (2) normal-appearing intestine; (3) ischemic-appearing liver; (4) normal-appearing liver

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VI. COMMENTS

No-injury control subject today, with the intent to measure foam volume recovery. The stated goal from previous procedures was recovery of a 3-4 L of foam from the pig's abdomen after one hour of observation. We really maxed out what we could inject today, taking care to bleed out all excess/free butane gas during and after all injections, and trying to fill up the abdomen as much as we could with foam only, including using a primary injection at t = 0, followed up by a secondary injection at t ~15 min (as a "top-off"). The extremes that we took to blow up the abdomen with foam were reflected in the very high IAP pressures we saw today (almost 50 mm Hg, something we would not want to see clinically). Despite all of these drastic measures, we really could not get much more than ~2.5 L of foam out of the abdomen after an hour. So the 3-4 L volume goal probably is not realistic. Importantly, though, we were able to maintain an IAP >10 mm Hg for most of the hour observation period during the 1st series of injections and throughout the entire period during the second series of injections. Since we accomplished this, I would say we are ready to move forward and start a trial of noncompressible injury with foam treatment.

Another important observation from today & recent procedures: the foam only occupies a space that is within a relatively short distance from the injector tip. The foam does not fill up every "nook and cranny" within the peritoneal cavity, like a gas would. In this model, the injected foam basically assumes a shape/size of a flattened American football (see Figs).

I think the important things that we have learned in the procedures over the past month are:
- The excess butane gas needs to be vented out of the abdomen during the injection.
- A secondary injection 10-15 min after the initial injection will help maintain the IAP stay over 10 mm Hg during the one hour observation period.
- It may be helpful to guide the tip of the injector into multiple areas during the injection, to aid in foam dispersion into a greater percentage of the potential space of the peritoneal cavity.

VII. PLAN

Return to the injury mechanism with calcium alginate foam treatment on Tue Mar 11th, beginning at 8 AM. We will use two subjects, using the standard noncompressible injury, and treated with the same formulation/mass/injection technique we used today (no biologics yet). If those procedures go well, then this could be the start of an actual trial of (1) foam *vs*. (2) foam + biologics *vs*. (3) no treatment in the noncompressible model.

Fig. 1, swine 200. Set-up for syringe pumps for infusion of CaCl2 solution into the alginate foam injection system. Four pumps (each using a 50 cc syringe) are used in parallel during each injection. Canister of *ex vivo* injected foam is sitting at left.

Fig. 2, swine 200. Appearance of swine after first series of foam injections completed, about 15 min into the 60 min observation period. IAP at this point was 42 mm Hg.
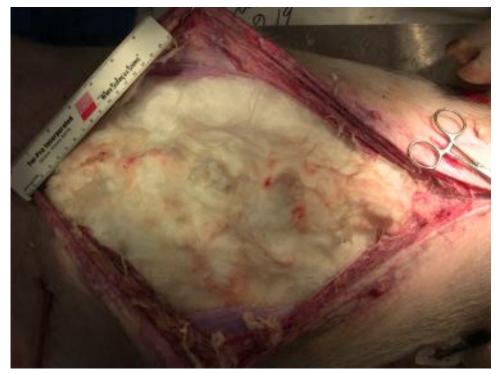
Fig. 3, swine 200. Appearance of abdomen at reopening 1 h after the first series of injections. There was no obvious pocket of butane. Cephalad is to the right.
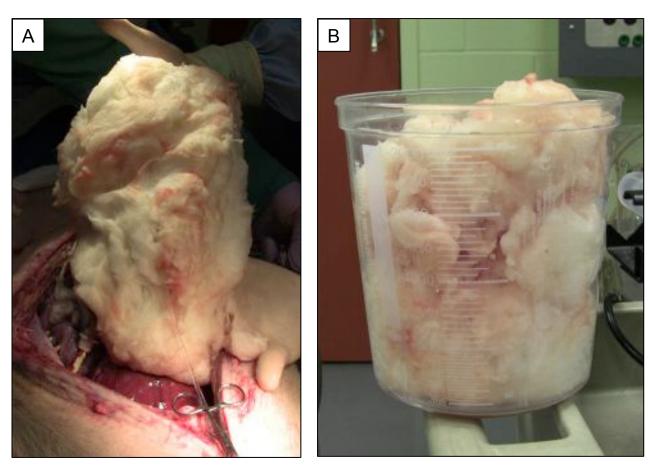
Fig. 4, swine 200. (A) Foam mass from Fig. 3 lifted out of the abdominal cavity as one intact specimen. (B) Volume of foam mass from first series of injections: 2+ L.

Figure 5, swine 200. Intraabdominal appearance after foam evacuation from the first series of injections. Overhead view of midline incision, retracted open; cephalad is to the right. Visceral surfaces were diffusely purplish and ischemic-looking.

Fig. 6, swine 200. Appearance of abdomen at reopening 1 h after the second series of injections. There was a small pocket of butane just under the incision (not visible). Cephalad is to the right.

Fig. 7, swine 200. (A) Foam mass from Fig. 6 lifted out of the abdominal cavity as one intact specimen. (B) Volume of foam mass from second series of injections: 2+ L.

I. OVERVIEW
Date: March 11, 2014
Swine no: 201
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega

II. PRE-INJURY PHASE

Start time: 8:08 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/28/2014
Pre-procedure wt: 39.6 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 145
- MAP: 110
- Temp: 34.6
- EtCO2: 27

Blood draw no. 1 (initial): 8:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:49 AM
Spleen wt: 561 gm
LR (22°C) infused after splenectomy: 1700 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 561 + 20 + 80 = 661 mL
- LR (22°C) infused (spleen replacement + incidental): 1700 + 520 = 2220 mL

Pre-injury VS
- HR: 161
- MAP: 107
- Temp: 32.5

- EtCO2: 26
- IAP: 0

III. INJURY & TREATMENT PHASE

3.8% alginate, Tween 20 = 0.6%, 21 mL/min x 4, 1.14 M $CaCl_2$.
500 recovered

Time of injury: 9:06 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.
Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).
Clotting factors: none.
Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began ~1 min after injury, after the abdomen had been completely closed with clips. Shortly after injection, there appeared to a malfunction of the syringe injection pump (Harvard Apparatus), which began making strained mechanical noises. The alginate reservoir was switched, and the foam injection continued. But there was a several min period in which there was no injection. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. A secondary injection was not performed because the IAP remained 15-20 mm Hg for the duration of the procedure.
Total mass foam + gas injected: 418 g
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 85
Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 9:07 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 9:21 AM

15 min post-injury VS
- HR: 200
- MAP: 30
- Temp: 34.6
- EtCO2: 10
- IAP: 25

Blood draw no. 3: (34 min post-injury): 9:40 AM

Final (34 min) VS

- HR: 109
- MAP: 15
- Temp: 34.5
- EtCO2: 0
- IAP: 15

Survival at 60 min? No
Target MAP attained? No
Time of death: 9:40 AM
Cause of death: exsanguination from liver injury prior to re-opening abdomen
Interval from injury to death: 34 min

Post-treatment fluid data:
- Blood loss 3202 mL (suction) + 773 mL (clot) = 3975 mL
- IV fluid given: LR (37°C): 4150 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & rock-hard/tense (IAP ~15 mm Hg). Upon re-opening abdomen, first thing visible was a thin layer of foam covering the viscera, with some blood tinge (see Figs). Exploring deeper into abdomen revealed a small amount of foam mixed with clot/blood in the right gutter (see Figs), and then a large amount of clot & blood in the deeper recesses. No toxic effects to the intraabdominal organs noted.
Volume foam recovered: ~500 mL
Heart: not examined.
Number of hepatic veins lacerated: 1 medium-sized, to LL lobe.
Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 882 g

Tissue harvested: snout for nasal membranes

VI. COMMENTS

Spleen was quite large for subject size; not sure if this meant anything. Subject also started out hypothermic, T ~34˚C, dropped to ~32˚C at time of injury, this likely affected subject's response to injury, clotting, etc.
Subject was markedly tachycardic after foam injection until shortly before death. Relatively rapid fatal course… not sure why. Also, only 0.5 L foam recovered, despite having IAP >15 mm Hg for duration of procedure.
See additional comments under swine 202.

VII. PLAN

Repeat these interventions in Swine no. 203 & 204 on Tue Mar 18th, 2014.

Figure 1, swine 201. Intraabdominal appearance immediately after swine expired from injury. Overhead view of midline incision, retracted open; cephalad is to the right. Amount of foam recovered ~500 mL.
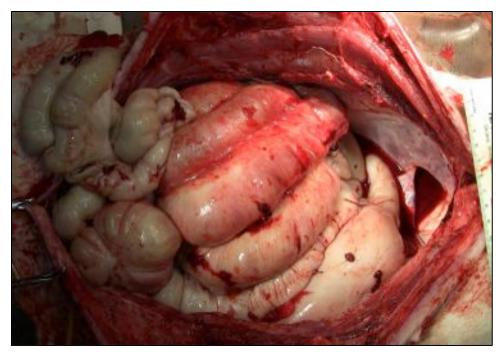
Figure 2, swine 201. Intraabdominal appearance from Fig. 1, after foam & liver were removed. Overhead view of midline incision, retracted open; cephalad is to the right. No obvious purplish discoloration to viscera.
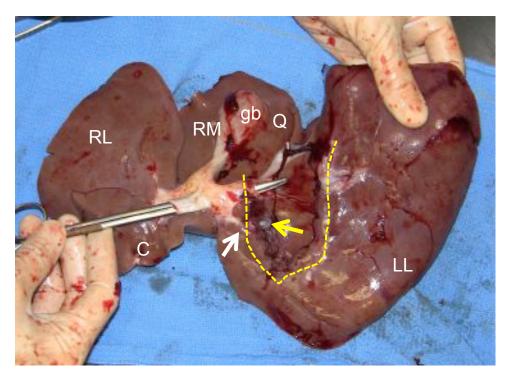
Figure 3, swine 201. Liver *ex vivo,* inferior aspect. Scissors has been inserted into the opened end of the portal vein, with the tips exiting out of the transected branch of PV to LL lobe. Yellow arrow indicates lumen of transected hepatic vein to LL lobe. White arrow indicates a secondary branch of the PV to the LL lobe that was cut. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.

I. OVERVIEW
Date: March 11, 2014
Swine no: 202
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam, new formulation
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega

II. PRE-INJURY PHASE

Start time: 10:10 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/28/2014
Pre-procedure wt: 40.2 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
　　1. Endotracheal tube with ETCO2 monitor
　　2. EKG clips
　　3. Left ear vein angiocath (20g) for supplemental LR
　　4. Right carotid artery angiocath (20g), cutdown; for BP monitor
　　5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
　　6. Transabdominal cystotomy for 16 Fr Foley catheter
　　7. Rectal temp probe
　　8. Pulse oximetry
　　9. Heating pad below subject
　　10. Intraabdominal pressure monitor

Initial VS
　　• HR: 108
　　• MAP: 118
　　• Temp: 38.2
　　• EtCO2: 31

Blood draw no. 1 (initial): 10:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:44 AM
Spleen wt: 338 gm
LR (22°C) infused after splenectomy: 1013 mL at 150 mL/min

Pre-injury fluid data:
　　• Blood loss (spleen weight + phlebotomies + incidental): 338 + 20 + 31 = 389 mL
　　• LR (22°C) infused (spleen replacement + incidental): 1013 + 110 = 1123 mL

Pre-injury VS
　　• HR: 151
　　• MAP: 116
　　• Temp: 36.4

- EtCO2: 15
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 10:58 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The nozzle of the foam injector was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip directed into the right colic gutter.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the nozzle in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the calcium began ~30 sec after injury, after the abdomen had been completely closed with clips. The calcium injection required ~2 min to complete. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. After injection completed, the nozzle was withdrawn and a final towel clip was placed in the space where the nozzle had been inserted. A secondary injection was not performed because the IAP remained >20 mm Hg for the duration of the procedure.

Total mass foam + gas injected: 602 g

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 95

Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 10:59 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): N/A

15 min post-injury VS
- HR: 186
- MAP: 36
- Temp: 35.7
- EtCO2: 11
- IAP: 30

Blood draw no. 3: (39 min post-injury): 11:37 AM

Final (40 min) VS
- HR: 145
- MAP: 14
- Temp: 33.1
- EtCO2: 0

- IAP: 24

Survival at 60 min? No
Target MAP attained? No
Time of death: 11:38 AM
Cause of death: exsanguination from liver injury prior to re-opening abdomen
Interval from injury to death: 40 min

Post-treatment fluid data:
- Blood loss 2993 mL (suction) + 1348 mL (clot) = 4341 mL
- IV fluid given: LR (37°C): 4145 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended & rock-hard/tense with final IAP ~20 mm Hg. Upon reopening incision, moderate rush of butane gas from superior portion of incision. Thin layer of foam covering intestines superficially, minimal blood tinge. Large amounts clotted & unclotted blood underneath. No obvious areas of discoloration of the intestines or other organs (see Figs).
Volume foam recovered: ~1200 mL
Heart: no emboli in any chamber; marked LV hypertrophy (see Figs).
Number of hepatic veins lacerated: one medium-sized vein to LL lobe.
Portal vein injury: 1 large branch, to LL lobe
Other: none
Ex vivo total liver wt: 840 g
Tissue harvested: none

VI. COMMENTS

   Similar result to no. 201: relatively rapid fatal course. So with two subjects in whom we have attempted to keep a high IAP with foam injection, the treatment appeared to be worse than the disease, i.e., the results with these two swine appear to have been worse than what we have seen before, either with the no-treatment controls or with a low-pressure (IAP <5 mm Hg) treatment. Obviously we have only done the high-pressure treatment with two swine, so these are not firm conclusions, but the preliminary results do not look good.
   My previous emphasis on getting 3-4 L volume of foam recovered does not appear possible or even relevant any more. Both subjects from today had modest foam recovery volumes (0.5 & 1.2 L), but their IAP's were mostly >20 and as high as 40 at some points. Another observation: the HR of these subjects goes up considerably with the IAP, sometimes >200 (but the MAP did not; a result of decreased venous return to the heart caused by the high IAP?).
   So pushing the IAP way up did not yield the hemostatic results I was hoping for… in fact, almost the opposite appears to be happening.
   So in no particular order of importance or relevance, here are my observations/thoughts to date:

- Pushing the IAP above 20 mm Hg in this model does not seem helpful, from N = 2.
- The injected foam does not mix with the shed blood, except right at the foam/blood interface. This may have implications for use of clotting factors, because if there is no mixing of the treatment with the blood, then any enhanced efficacy from added clotting factors may not be measureable.

- Pushing the IAP up appears to limit the volume of foam that is recovered. It may be that the butane is causing most of the distension, even though we are venting off the excess butane as much as possible. Can the rate of butane flow be limited? Could we try an alternative gas?
- The subjects develop tachycardia ≥200 bpm with high IAP. This may be a result of obstructed venous return to the heart, or some autonomic phenomena, or something else, I'm not sure.
- On the other hand, the high IAP may somehow be augmenting the pressure in the portal system, thereby increasing portal hemorrhage.
- The pressure within the abdomen may not be uniformly distributed. That is, we probably should not assume that the abdomen is like a big balloon, whereby increasing the pressure at one location within the balloon results in a rapid and equal pressure increase everywhere in the balloon. So an IAP of 40 mm Hg which is detected by our intraabdominal IV bag in the left paracolic gutter may not reflect the pressure at the liver injury site.
- We should keep closer tabs on the subjects body temp and pCO_2, because these were out of range in one or both subjects today and might have contributed to the suboptimal outcomes (but I doubt temp & pCO_2 were primary issues).
- Injection of the alginate foam in only one region of the abdomen (i.e., the right paracolic gutter) may never be effective. Perhaps if the foam was injected simultaneously in multiple regions, then the effect would be more pronounced. But multi-region injection may not be practical. We could try injection of foam right at the injury site again, but this would risk cardiac embolism.
- Another group (David King's at MGH) already has demonstrated efficacy in treatment of a (different) porcine noncompressible hemorrhage model with an expansile urethane foam. So in theory, a tamponade foam should have some efficacy. But (1) their model is markedly different from ours, and (2) their foam is markedly different from ours. One possibility that we might be dealing with is that we are either using the wrong model or the wrong foam (or both)… but we simply don't (and can't) know at this point.

At this time, I actually do not know the best route to move forward with this model. At a minimum, we probably should repeat today's treatments (i.e., using a high IAP goal) to get an N = 4 before making firmer conclusions.

VII. PLAN

Repeat these interventions in Swine no. 203 & 204 on Tue Mar 18th, 2014.

Figure 1, swine 202. Side view of subject after preparation (including splenectomy), but prior to injury. The midline incision has been closed with towel clips. Cephalad is to the right.
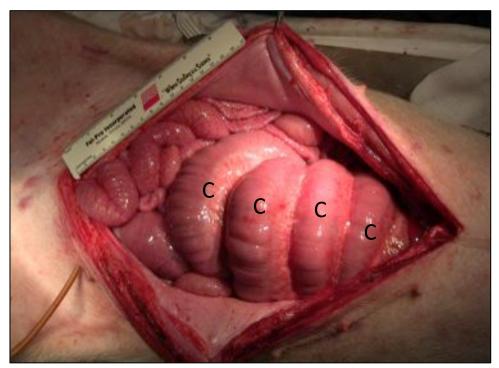

Figure 2, swine 202. Same time point as in Fig. 1, but overhead view of opened midline incision to show appearance of viscera. Primarily visible is the "spiral" formation of the colon. Cephalad is to the right.

Figure 3, swine 202. Side view of subject about 15 min after injury; IAP ~30 mm Hg. Cephalad is to the right. Compare degree of abdominal distension with Fig. 1.

Figure 4, swine 202. Intraabdominal appearance immediately after swine expired from injury. Overhead view of midline incision, retracted open; cephalad is to the right. Amount of foam recovered ~1,200 mL.

Figure 5, swine 202. Appearance of left ventricle bivalved (cut in cross section). LV wall was thickened, significance unknown. Heart chambers were without embolus.
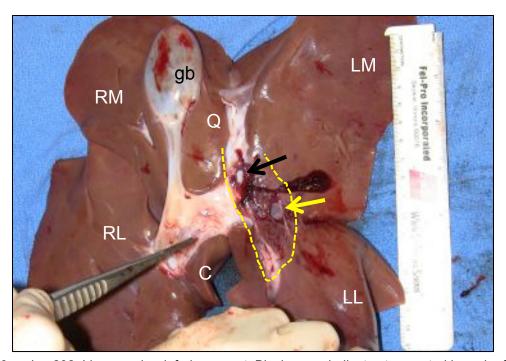
Figure 6, swine 202. Liver *ex vivo,* inferior aspect. Black arrow indicates transected branch of PV to LL lobe. Yellow arrow indicates lumen of transected hepatic vein to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.
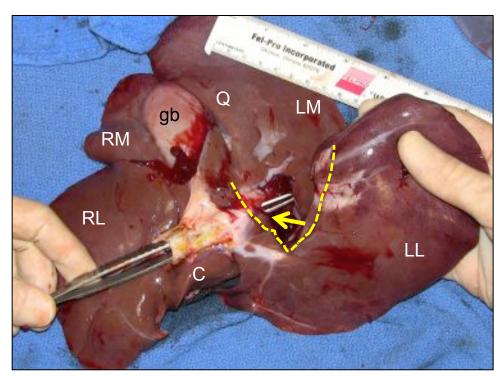
I. OVERVIEW
Date: March 18, 2014
Swine no: 203
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam (new triple injector system)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega

II. PRE-INJURY PHASE

Start time: 8:05 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/28/2014
Pre-procedure wt: 38.0 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 135
- MAP: 111
- Temp: 37.5
- EtCO2: 25

Blood draw no. 1 (initial): 8:17 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:42 AM
Spleen wt: 310 gm
LR (22°C) infused after splenectomy: 930 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 310 + 20 + 10 = 340 mL
- LR (22°C) infused (spleen replacement + incidental): 930 + 105 = 1035 mL

Pre-injury VS
- HR: 136
- MAP: 108
- Temp: 35.9

- EtCO2: 24
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 9:01 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The three Tygon tubes of the foam triple-injector were inserted between the towel clips through the lower part of the midline incision and into the abdomen (see Figs from subject# 204), with the tips directed into the right upper, left upper, and left lower quadrants.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. A second foam injection was performed at t = 7 min.

Total mass (foam + gas) injected: 610 g

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 85

Resuscitation fluid: warm LR (3.8 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 9:02 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 9:16 AM

15 min post-injury VS
- HR: 126
- MAP: 89
- Temp: 35.6
- EtCO2: 23
- IAP: 5

Blood draw no. 3: (60 min post-injury): 10:01 AM

Final (60 min) VS
- HR: 132
- MAP: 40
- Temp: 35.9
- EtCO2: 24
- IAP: ~5 (IAP monitor was malfunctioning; this is an estimate)

Survival at 60 min? Yes
Target MAP attained? Briefly
Time of death: 10:07 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 66 min

Post-treatment fluid data:
- Blood loss 1721 mL (suction) + 244 mL (clot) = 1965 mL
- IV fluid given: LR (37°C): 4070 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, not very tense (IAP ~5 mm Hg, but IAP monitor
    was malfunctioning in this subject). Upon re-opening abdomen, first thing visible was foam covering the
    viscera, with some blood tinge (see Figs). Foam had peanut-shape configuration. Exploring deeper into
    abdomen revealed a small amount of foam mixed with clot/blood in the deeper recesses. Small area of
    intestinal ischemia noted. Of note, there was a large clot covering the injury site (see Figs). Animal still alive
    during exploration and foam/blood/clot evacuation.
Volume foam recovered: ~2,000 mL
Heart: not examined.
Number of hepatic veins lacerated: 1 very large, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 704 g

Tissue harvested: snout for nasal membranes

VI. COMMENTS

       Today we tried the triple-injector for foam delivery for the first time. Foam recipe was the same, but
qualitatively the foam seemed different. During injection, the rush of gas that continually needed to be evacuated
was not as noticeable as it has been in recent memory. Consistent with this observation, we did not have large
increase in IAP as has been seen previously; we only got up to 5 mm Hg today. And at necropsy, the foam
morphology looked like "peanuts." The foam morphology in previous subjects was pretty much amorphic. One
result which I was hoping to see but which did not occur was an increase in foam volume recovered. We extracted
~2 L of foam from today's subject, which has been about the max volume we have been able to recover. So
injecting at multiple intraabdominal sites did not seem to increase the foam volume.
       Today's animal survived. There was a large clot covering the injury site, effectively staunching the
hemorrhage from the HV & PV injuries. Whether this clot was a coincidence, or somehow a result of the different
treatment apparatus, I don't know.

VII. PLAN

       See comments under Swine 204.

Figure 1, swine 203. Side view of subject after preparation (including midline incision & splenectomy), but prior to injury, showing relaxed state of the abdomen. Cephalad is to the right.
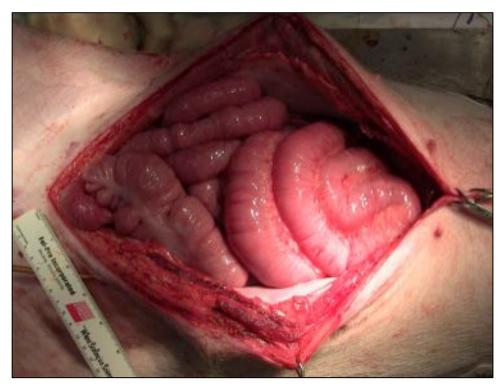

Figure 2, swine 203. Same time point as in Fig. 1, but overhead view of opened midline incision to show appearance of viscera. Primarily visible is the "spiral" formation of the colon (C). Cephalad is to the right.

Figure 3, swine 203. Foam injector set-up. (A) Canister. (B) Two syringe pump set-ups (four syringes per pump, for parallel injection of calcium chloride).
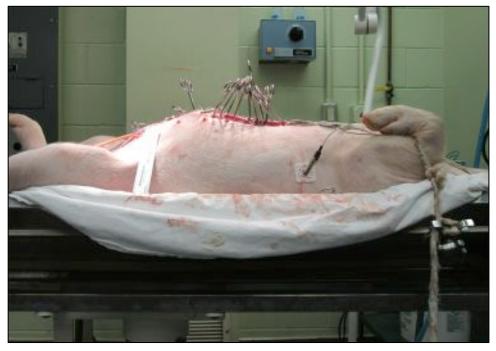
Figure 4, swine 203. Side view of subject about 30 min after injury; IAP ~5 mm Hg. Cephalad is to the right. Compare degree of abdominal distension with Fig. 1.

Figure 5, swine 203. Intraabdominal appearance immediately 60 min after injury. Overhead view of midline incision, retracted open; cephalad is to the right. Amount of foam recovered ~2 L. Note "peanut" configuration of foam compared to previous subjects, in which foam was more amorphous.
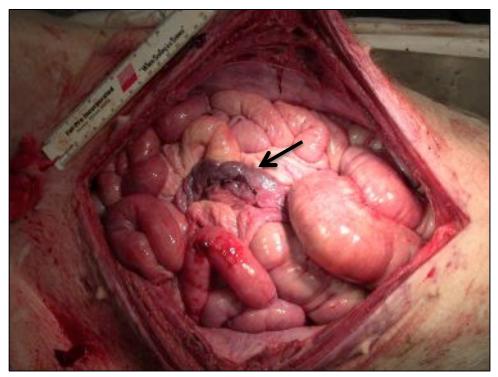
Figure 6, swine 203. Intraabdominal appearance after euthanasia. Overhead view of midline incision, retracted open; cephalad is to the right. Foam and liver have been removed. Some ischemic-appearing intestine in the central area (arrow).
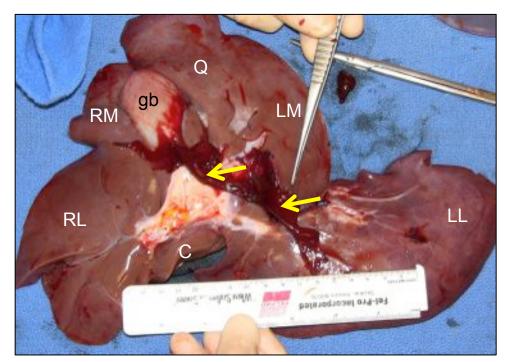
Figure 7, swine 203. Liver *ex vivo,* inferior aspect. Yellow arrows large clot that was covering injury site. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.
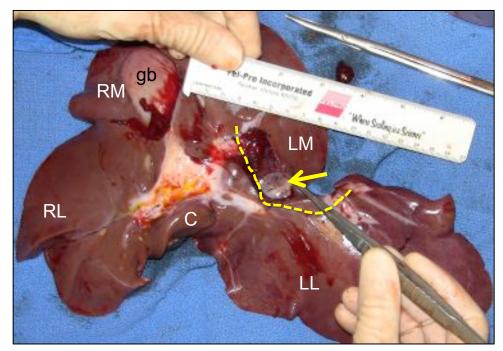

Figure 8, swine 203. Liver *ex vivo,* inferior aspect. Clot in Fig. 7 has been removed. Yellow arrow indicates very large lumen of transected hepatic vein to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; gb = gallbladder.

Figure 9, swine 203. Liver *ex vivo,* inferior aspect. Forceps placed through the transected branch of PV to LL lobe (yellow arrow). Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.

I. OVERVIEW
Date: March 18, 2014
Swine no: 204
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam (new triple injector system)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega

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II. PRE-INJURY PHASE

Start time: 10:34 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 02/28/2014
Pre-procedure wt: 38.4 kg

Anesthetic Induction: Telazol (4.4 mg/kg), Ketamine (2.2 mg/kg), Xylazine (2.2 mg/kg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 150
- MAP: 106
- Temp: 38.7
- EtCO2: 39

Blood draw no. 1 (initial): 11:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 11:15 AM
Spleen wt: 314 gm
LR (22°C) infused after splenectomy: 950 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 314 + 20 + 42 = 376 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 55 = 1055 mL

Pre-injury VS
- HR: 123
- MAP: 107
- Temp: 37.0

- EtCO2: 42
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 11:33 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The three Tygon tubes of the foam triple-injector were inserted between the towel clips through the lower part of the midline incision and into the abdomen (see Figs), with the tips directed into the right upper, left upper, and left lower quadrants. The length of each of the Tygon tubes was ~20 cm (about 2x longer than used in swine 203).

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: none.

Technique: with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. Only one injection was performed, but this used two canisters. The level of abdominal distension appeared adequate.

Total mass (foam + gas) injected: 625 g

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 85

Resuscitation fluid: warm LR (3.8 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 11:34 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 11:48 AM

15 min post-injury VS
- HR: 186
- MAP: 39
- Temp: 36.4
- EtCO2: 26
- IAP: 5

Blood draw no. 3: (37 min post-injury): 12:10 PM

Final (36 min) VS
- HR: 150
- MAP: 12
- Temp: 37.4
- EtCO2: 0
- IAP: 14 (IAP monitor accurate? Abdomen was not tense)

Survival at 60 min? No
Target MAP attained? Less than 1 min early on
Time of death: 12:12 AM
Cause of death: exsanguination from injury
Interval from injury to death: 39 min

Post-treatment fluid data:
- Blood loss 3058 mL (suction) + 586 mL (clot) = 3944 mL
- IV fluid given: LR (37°C): 3935 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, not very tense (IAP ~5 mm Hg, not sure if IAP monitor was malfunctioning in this subject). Upon re-opening abdomen, unclotted blood was at surface, with foam underneath. Less foam coverage of intestines than in #203. Foam had peanut-shape configuration, similar to #203 (see Figs). Exploring deeper into abdomen revealed a more foam mixed with clot/blood in the deeper recesses. No intestinal ischemia noted. Of note, there was a small clot partially covering the injury site (see Figs).
Volume foam recovered: ~2,000 mL
Heart: not examined.
Number of hepatic veins lacerated: 1 medium, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 791 g

Tissue harvested: snout for nasal membranes

VI. COMMENTS

2nd attempt with triple-injector foam delivery. Similar findings as with #203 (refer to that description): peanut-shaped foam morphology; not much in way of gaseous distension; relatively low IAP; a clot (small) covering injury site. We still could not get more than 2 L of foam recovery.

This subject died at ~40 min. Similar to #203, it had a clot at the injury site, though smaller. Don't know the significance of this in the present context. The clot did not prevent the animal from bleeding to death.

Qualitatively, the triple-injector system behaves differently and maybe has different (better?) efficacy than our single injector tube system. Today's results did not confirm last week's failures. I guess we should stick with the triple-system for now. Perhaps we can try adding in clotting factors for the next two subjects, and see what happens.

VII. PLAN

On the next three consecutive Tuesdays (Mar 25, Apr 1, Apr 8), we will be injuring swine for the survival portion of the NEDED studies. On Tue Apr 15th, I would like to test foam + clotting factors in two swine with noncompressible injury.

Figure 1, swine 204. Side view of subject after preparation (including midline incision & splenectomy), but prior to injury, showing relaxed state of the abdomen. Cephalad is to the right.

Figure 2, swine 204. Same time point as in Fig. 1, but overhead view of opened midline incision to show normal appearance of viscera. Cephalad is to the right.
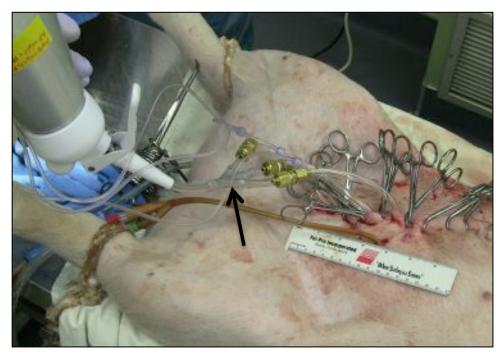
Figure 3, swine 204. Foam injector set-up, inserted into the abdomen, just prior to injury & injection. Left oblique view of the pelvic region, showing lower half of the midline incision (which is closed with towel clips). Note the trifurcation branch (arrow) of the injector tubing, leading to three separate tube that pass through the incision and deliver foam to different areas of the abdomen.
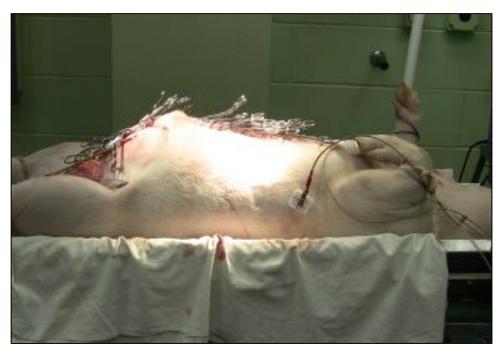
Figure 4, swine 204. Side view of subject about 15 min after injury; IAP ~5 mm Hg. Cephalad is to the right. Compare degree of abdominal distension with Fig. 1.
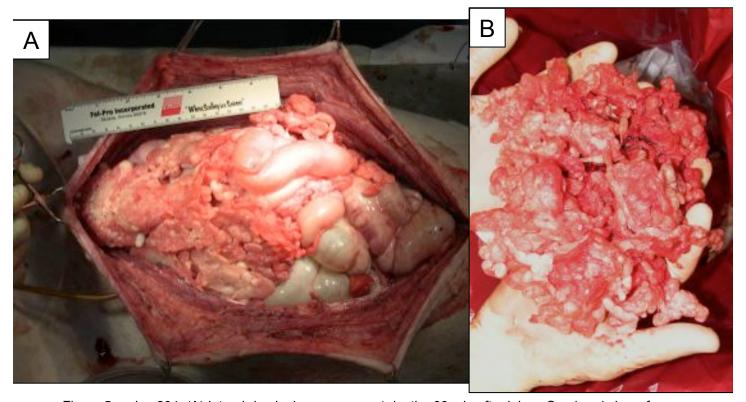
Figure 5, swine 204. (A) Intraabdominal appearance at death ~30 min after injury. Overhead view of midline incision, retracted open; cephalad is to the right. Amount of foam recovered ~2 L. Note "peanut" configuration of foam compared to previous subjects, in which foam was more amorphous. (B) Close-up of foam after extraction from abdomen.
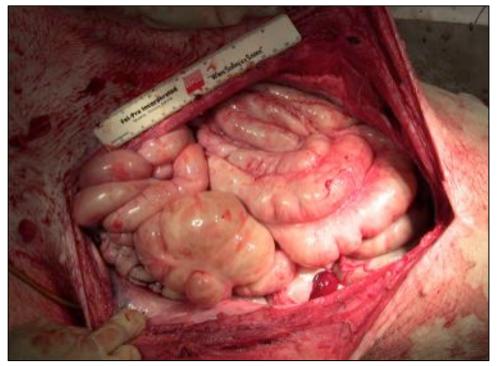
Figure 6, swine 204. Intraabdominal appearance after death. Overhead view of midline incision, retracted open; cephalad is to the right. Foam and liver have been removed.
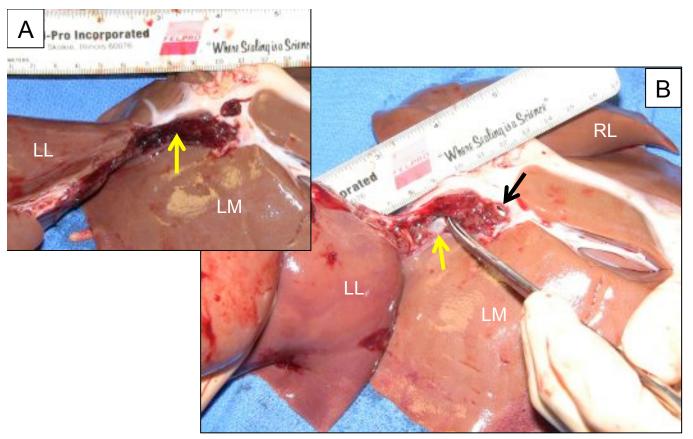
Figure 7, swine 204. Liver *ex vivo,* inferior left oblique aspect, showing injury site. (A) Yellow arrow indicates a small clot that was covering injury site. (B) Clot removed; yellow arrow indicates open lumen of HV branch to LL lobe; black arrow = lumen of cut PV branch to LL lobe. RL = right lateral lobe; LM = left medial lobe; LL = left lateral lobe.
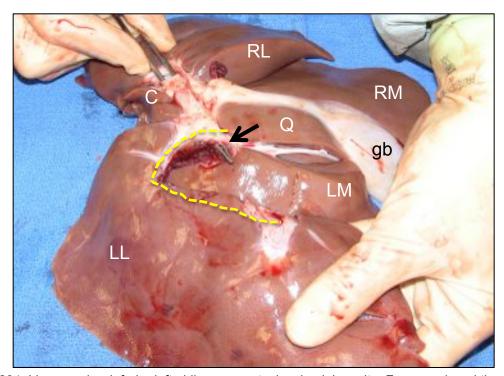
Figure 8, swine 204. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Forceps placed through the cut branch of PV to LL lobe (arrow). Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe; gb = gallbladder.

I. OVERVIEW
Date: April 15, 2014
Swine no: 211
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 8:05 AM
Swine sex: female (gilt)
Date swine received from UNL Mead: 04/04/2014
Pre-procedure wt: 39.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
  1. Endotracheal tube with ETCO2 monitor
  2. EKG clips
  3. Left ear vein angiocath (20g) for supplemental LR
  4. Right carotid artery angiocath (20g), cutdown; for BP monitor
  5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
  6. Transabdominal cystotomy for 16 Fr Foley catheter
  7. Rectal temp probe
  8. Pulse oximetry
  9. Heating pad below subject
  10. Intraabdominal pressure monitor

Initial VS
  • HR: 68
  • MAP: 120
  • Temp: 37.5
  • EtCO2: 34

Blood draw no. 1 (initial): 8:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:38 AM
Spleen wt: 257 gm
LR (22°C) infused after splenectomy: 770 mL at 150 mL/min

Pre-injury fluid data:
  • Blood loss (spleen weight + phlebotomies + incidental): 257 + 20 + 48 = 325 mL
  • LR (22°C) infused (spleen replacement + incidental): 770 + 150 = 920 mL

Pre-injury VS
  • HR: 94
  • MAP: 107
  • Temp: 36.9

- EtCO2: 37
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:57 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip initially directed into the right upper quadrant.
Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).
Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.
Technique: with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.
Total mass injected: 288 mL $CaCl_2$, 805 g alginate.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 87
Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 8:59 AM (within 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 9:12 AM

15 min post-injury VS
- HR: 166
- MAP: 47
- Temp: 37.0
- EtCO2: 18
- IAP: 10

Blood draw no. 3: (60 min post-injury): 10:01 AM

Final (30 min) VS
- HR: 160
- MAP: 10
- Temp: 37.0
- EtCO2: 5
- IAP: 10

Survival at 60 min? No
Target MAP attained? No
Time of death: 9:28 AM
Cause of death: exsanguination from injury
Interval from injury to death: 31 min

Post-treatment fluid data:
- Blood loss 2380 mL (suction) + 544 mL (clot) + 269 mL (lap pads) = 3193 mL
- IV fluid given: LR (37°C): 4255 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~10 mm Hg). Upon re-opening abdomen, nonclotted blood surged out; foam covering the viscera (see Figs). Foam had peanut-shape configuration, mixed with blood (see Figs). Large amount clotted & unclotted blood. No obvious intestinal ischemia noted.
Volume foam recovered: 2+ L (see Figs)
Heart: RV & RA without evidence of ischemia.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 916 g

Tissue harvested: none

VI. COMMENTS

No obvious treatment effect, subject dead within 30 min. Injury was good.

VII. PLAN

Repeat in Swine 212.

Figure 1, swine 211. Pre-injury; overhead view of opened midline incision to show normal appearance of viscera. Cephalad is to the right.
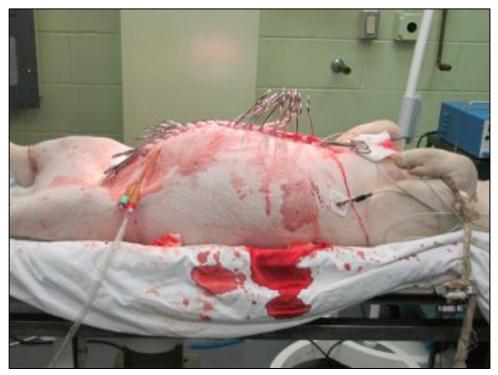
Figure 2, swine 211. Side view of subject about 15 min after injury; IAP ~10 mm Hg. Cephalad is to the right. This subject expired ~30 min after injury.
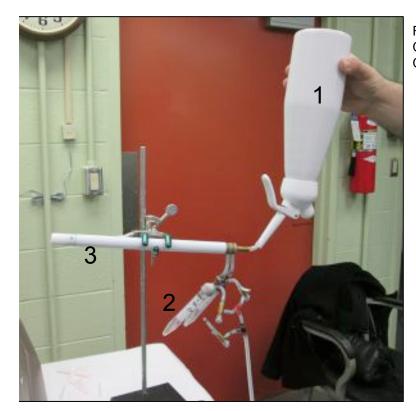
Figure 3, swine 211. Injection apparatus. (1) Commercial creamer container for alginate. (2) Conical tube containing biologics. (3) Injector nozzle.

Figure 4, swine 211. Foam recovered from abdomen after subject expiration. Volume = 2+ L. Mixed with clotted & unclotted blood.

Figure 5, swine 211. Evacuation of foam, clots, and blood after expiration. Cephalad is at upper right.

Figure 6, swine 211. Examination of right ventricle of heart at necropsy. No evidence of embolism.
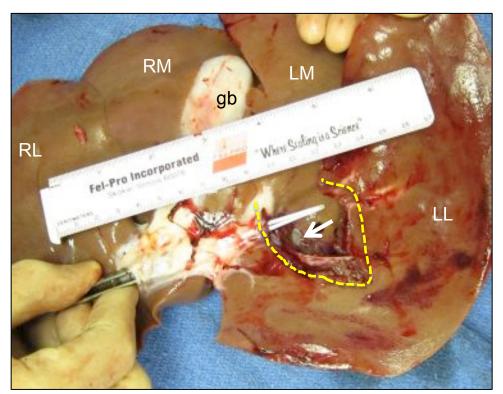
Figure 7, swine 211. Liver *ex vivo,* inferior aspect, showing injury site. Forceps placed through the cut branch of PV to LL lobe (arrow). Dashed yellow line = gap in LL lobe induced by cut. White arrow = orifice of cut HV to LL lobe. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; gb = gallbladder.

I. OVERVIEW
Date: April 15, 2014
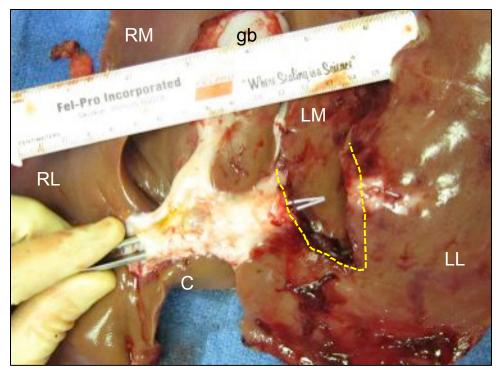Swine no: 212
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 10:15 AM
Swine sex: female (gilt)
Date swine received from UNL Mead: 04/04/2014
Pre-procedure wt: 40.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
 1. Endotracheal tube with ETCO2 monitor
 2. EKG clips
 3. Left ear vein angiocath (20g) for supplemental LR
 4. Right carotid artery angiocath (20g), cutdown; for BP monitor
 5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
 6. Transabdominal cystotomy for 16 Fr Foley catheter
 7. Rectal temp probe
 8. Pulse oximetry
 9. Heating pad below subject
 10. Intraabdominal pressure monitor

Initial VS
 • HR: 86
 • MAP: 116
 • Temp: 38.4
 • EtCO2: 38

Blood draw no. 1 (initial): 8:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:30 AM
Spleen wt: 304 gm
LR (22°C) infused after splenectomy: 915 mL at 150 mL/min

Pre-injury fluid data:
 • Blood loss (spleen weight + phlebotomies + incidental): 304 + 20 + 50 = 374 mL
 • LR (22°C) infused (spleen replacement + incidental): 915 + 70 = 985 mL

Pre-injury VS
 • HR: 82
 • MAP: 124
 • Temp: 37.4

- EtCO2: 38
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 11:03 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip initially directed into the right upper quadrant.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.

Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 272 mL $CaCl_2$, 661 g alginate.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 100

Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 11:04 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 11:18 AM

15 min post-injury VS
- HR: 105
- MAP: 71
- Temp: 37.5
- EtCO2: 32
- IAP: 15

Blood draw no. 3: (60 min post-injury): 12:03 PM

Final (60 min) VS
- HR: 140
- MAP: 41
- Temp: 37.4
- EtCO2: 30
- IAP: 15

Survival at 60 min? Yes
Target MAP attained? No
Time of death: 12:10 PM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 67 min

Post-treatment fluid data:
- Blood loss 1456 mL (suction) + 300 mL (clot) + 82 mL (lap pads) = 1838 mL
- IV fluid given: LR (37°C): 4330 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~15 mm Hg). Upon re-opening abdomen, foam covering the viscera (see Figs). Foam had peanut-shape configuration, mixed with blood (see Figs). Moderate amount clotted & unclotted blood. No obvious intestinal ischemia noted. Clot was covering injury site (see Figs).
Volume foam recovered: 2+ L (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 837 g

Tissue harvested: none

VI. COMMENTS

Subject survived injury fairly easily to 1 h. Injury was good, should have been lethal. Clot covering injury site (no foam in vicinity). Encouraging result, hopefully represents treatment effect. More data will tell.
Some changes I will be making in near future:

- Extend observation period to 3 h (IACUC approval pending).
- Lab testing (all except TEG) at these time points: pre-injury, and then 10, 30, 60, 90, 120, 150, and 180 min after injury.
- Include lactate level with lab tests
- TEG: pre-injury, 10 min after injury, and then at 180 min or death (whichever first).
- Continuous plotting of IAP, MAP & HR for every subject (this data captured every few seconds with our Bionet monitor). Ujwal to do.

VII. PLAN

Repeat alginate + FS foam Rx as done today in Swine 213 & 214 on Tue Apr 22nd, 8 AM.
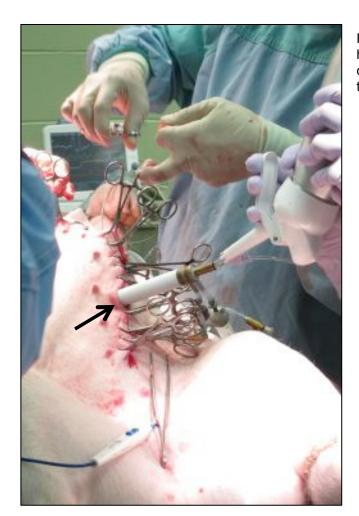
Figure 1, swine 212. Injection procedure. View toward the head. Injury has just been created, and upper incision is being closed with towel clips. Injector nozzle (arrow) was inserted through lower portion of incision prior to injury creation.

Figure 2, swine 212. Injection procedure. View toward the head. Incision has been closed, and injection proceeding.

Figure 3, swine 212. Side view of subject about 15 min after injury; IAP ~15 mm Hg. Cephalad is to the right. This subject survived the 1 h observation period.

Figure 4, swine 212. Overhead view of the re-opened abdomen after the 1 h observation period. Subject alive with MAP ~40. Cephalad to the right. Lobulated foam stained with blood is visible.

Figure 5, swine 212. Overhead view of the re-opened abdomen after the 1 h observation period and evacuation of foam/clots/blood. Subject still alive but MAP decreasing to ~20.
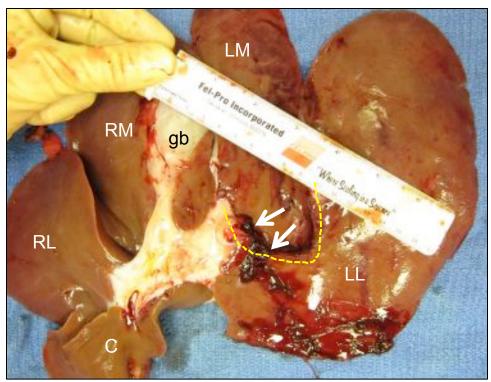
Figure 6, swine 212. Liver *ex vivo,* inferior aspect, showing injury site. Note clot overlying cut edge of liver (white arrows), covering transected HV/PV. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; gb = gallbladder.

Figure 7, swine 212. Liver *ex vivo,* inferior aspect, showing injury site. Clot in Fig. 6 has been removed, exposing cut vessels. Forceps has been inserted into transected PV to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; gb = gallbladder.

I. OVERVIEW
Date: April 22, 2014
Swine no: 213
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 08:04 AM
Swine sex: Male (barrow)
Date swine received from UNL Mead: 04/18/2014
Pre-procedure wt: 41.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 143
- MAP: 147
- Temp: 38.2
- EtCO2: 36

Blood draw no. 1 (initial): 8:34 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:01 AM
Spleen wt: 508 gm
LR (22°C) infused after splenectomy: 1600 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 518 + 20 + 201.9 = 739.9 mL
- LR (22°C) infused (spleen replacement + incidental): 1600 + 25 = 1625 mL

Pre-injury VS
- HR: 102
- MAP: 83
- Temp: 36.5

- EtCO2: 30
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:20 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip initially directed into the right upper quadrant.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.

Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 240 mL $CaCl_2$, 652.6 g alginate.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 65

Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 09:21 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

10 min post-injury VS
- HR: 122
- MAP: 43
- Temp: 36.6
- EtCO2: 21
- IAP: 10

Blood draw no. 2: (17 min post-injury): 09:37 AM

Final (17 min) VS
- HR: 70
- MAP: 17
- Temp: 36.6
- EtCO2: Apnea
- IAP: 10

Survival at 60 min? No
Target MAP attained? No

Time of death: 9:37 AM
Cause of death: exsanguination + foam/clot emboli. Clots found in the Right atrium and right ventricle
Interval from injury to death: 17 min

Post-treatment fluid data:
- Blood loss 2279.2 mL (suction) + 1083.6 mL (clot) + 119.6 mL (lap pads) = 3482.4 mL
- IV fluid given: LR (37°C): 2600 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~10 mm Hg). Upon re-opening abdomen, foam covering the viscera. Foam had peanut-shape configuration, mixed with blood. Large amounts of clotted & unclotted blood. No obvious intestinal ischemia noted. Large amount bubbles out of RV when opened. Clots along with foam is seen in the right ventricle and stringy clots in the right atrium at autopsy (see Figs).
Volume foam recovered: 2+ L
Heart: Foam & clot. See Figs.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 994.7 g

Tissue harvested: urine & nasal swabs (for Pulmonary Lab)

VI. COMMENTS

Subject 213 was relatively hypotensive prior to injury (MAP 80); expired at 17 min from combined bleeding & embolism.

VII. PLAN

Repeat in next subject, swine 214.
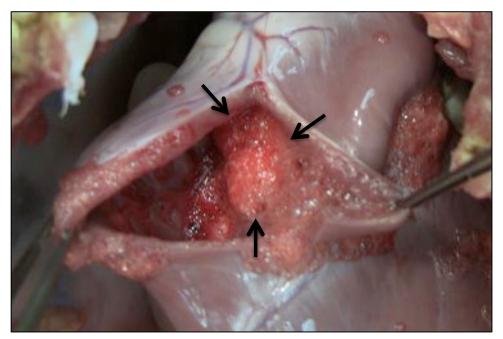
Figure 1, swine 213. Necropsy. Opening of right ventricle, showing embolus of foam + gas bubbles (arrows). Forceps are distracting walls of RV laterally. A much larger volume of bubbles than that shown in the image flowed out of the RV upon incision.
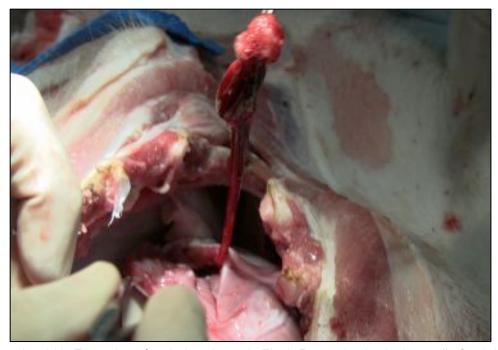
Figure 2, swine 213. Extraction of embolus shown in Fig 1. Foam plug had a long tail of red thrombus attached. Embolus is being pulled out with a forceps, which is just visible at the top of the image.
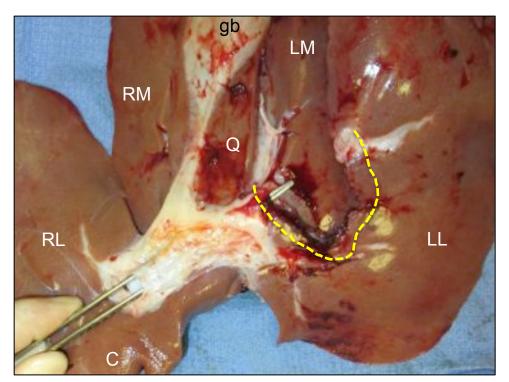
Figure 3, swine 213. Liver *ex vivo,* inferior aspect, showing injury site. Tips of forceps emerge from cut PV branch to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; gb = gallbladder.

I. OVERVIEW
Date: April 22, 2014
Swine no: 214
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Heimann, Fatemi, Noriega, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 10:24 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 04/18/2014
Pre-procedure wt: 42.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 99
- MAP: 107
- Temp: 39.0
- EtCO2: 41

Blood draw no. 1 (initial): 10:38 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 11:06 AM
Spleen wt: 393.9 gm
LR (22°C) infused after splenectomy: 1200 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 393.9 + 20 + 70 = 483.9 mL
- LR (22°C) infused (spleen replacement + incidental): 1200 + 175 = 1375 mL

Pre-injury VS
- HR: 88
- MAP: 125
- Temp: 37.5

- EtCO2: 37
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 11:17 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip initially directed into the right upper quadrant.
Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).
Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.
Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.
Total mass injected: 448 mL $CaCl_2$, 712.7 g alginate.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR (4.0 L preset maximum, or 100 mL/kg)
Time resuscitation fluid began: 11:18 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 11:27AM

15 min post-injury VS
- HR: 144
- MAP: 33
- Temp: 37.4
- EtCO2: 16
- IAP: 8

Blood draw no. 3: (30 min post-injury): 11:47 AM

Final (30 min) VS
- HR: 61
- MAP: 12
- Temp: 35.3
- EtCO2: Apnea
- IAP: 16

Survival at 60 min? No
Target MAP attained? No
Time of death: 11:47 AM
Cause of death: exsanguination from injury
Interval from injury to death: 30 min

Post-treatment fluid data:
- Blood loss 3855.5 mL (suction) + 566.2 mL (clot) + 213.1 mL (lap pads) = 4634.8 mL
- IV fluid given: LR (37°C): 4250 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~16 mm Hg). Upon re-opening abdomen, foam covering the viscera (see Figs). Foam had peanut-shape configuration, mixed with blood (see Figs). Moderate amount clotted & large amount of unclotted blood. No obvious intestinal ischemia noted. Clot was present close to but not covering injury site (see Figs).
Volume foam recovered: 2+ L (see Figs)
Heart: small red clot in RV; no foam or gas (see Figs)
Number of hepatic veins lacerated: 1, at the confluence of branches from LL and LM lobe (large injury)
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 910.2 g

Tissue harvested: none

VI. COMMENTS

Subject 214 expired at 30 min. Normotensive prior to injury (MAP 125). Cause of death = exsanguination. No evidence of treatment efficacy today with subjects 213 or 214.

VII. PLAN

Repeat in one swine on Tue Apr 29th, starting ~8 AM.
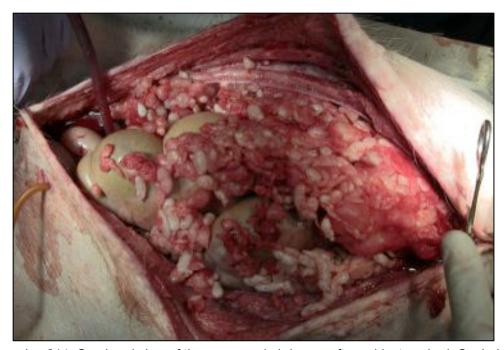
Figure 1, swine 214. Overhead view of the re-opened abdomen after subject expired. Cephalad to the right. Lobulated foam stained with blood is visible.
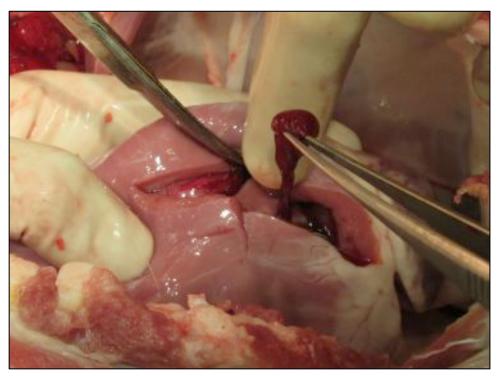
Figure 2, swine 214. Necropsy, opening of right ventricle *in situ*, demonstrating presence of small red clot without foam or gas bubbles.
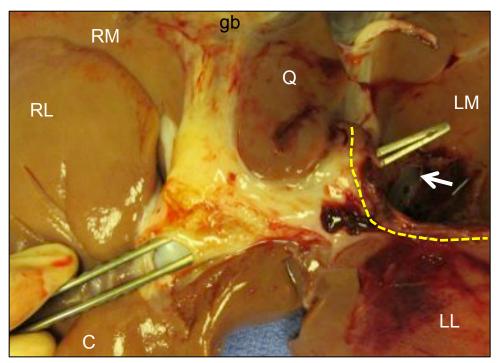
Figure 3, swine 214. Liver *ex vivo,* inferior aspect, showing injury site. Tips of forceps emerges from cut PV branch to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. White arrow = orifice of cut HV to LM lobe (confluence injury in this subject). RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; gb = gallbladder.

I. OVERVIEW
Date: April 29, 2014
Swine no: 215
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Fatemi, Noriega, Ismail, Vanderslice


II. PRE-INJURY PHASE

Start time: 8:07AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 4/18 /2014
Pre-procedure wt: 30.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 55
- MAP: 94
- Temp: 36.2
- EtCO2: 15

Blood draw no. 1 (initial): 08:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 301.5 gm
LR (22°C) infused after splenectomy: 904.5mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 301.5+ 30 + 11.7 = 343.2 mL
- LR (22°C) infused (spleen replacement + incidental): 904.5 + 150 = 1054.5 mL

Pre-injury VS
- HR: 45
- MAP: 85
- Temp: 33.5

- EtCO2: 11
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:19 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted between the towel clips through the lower part of the midline incision and into the abdomen, with the tip initially directed into the right upper quadrant.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.

Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to the lower quadrants of the abdomen; injection near the injury was avoided.

Total mass injected: 240 mL $CaCl_2$, 627.7g alginate.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 70

Resuscitation fluid: warm LR (3.1 L preset maximum, or 100 mL/kg)

Time resuscitation fluid began: 09:19 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:29 AM

15 min post-injury VS
- HR: 91
- MAP: 97
- Temp: 33.3
- EtCO2: 11
- IAP: 11

Blood draw no. 3: (30 min post-injury): 09:49 AM

Final (60 min) VS
- HR: 56
- MAP: 79
- Temp: 32.4
- EtCO2: 10
- IAP: 14

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 10:26
Cause of death: exsanguination from euthanasia
Interval from injury to death: 67 min

Post-treatment fluid data:
- Blood loss 294.8 mL (suction) + 30 mL (clot) + 660.9 mL (lap pads) = 985.7 mL
- IV fluid given: LR (37°C): 650 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, moderately tense (IAP ~15 mm Hg). Upon re-opening abdomen, foam covering the viscera (see Figs). Foam had peanut-shape configuration, not mixed with blood (see Figs). Minimal amounts of unclotted blood and a big clot was seen on the superior surface of the liver, and also covering the injury site (see Figs). The HV to the LL lobe was small, and was compressed flat (i.e., occluded). Purplish discoloration of the small bowel present (see Figs).
Volume foam recovered: 2+ L (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1 small, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 575.1g

Tissue harvested: injury site clot for FI immunohistochemistry

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VI. COMMENTS

No. 215 gave us curious results. In the days leading up to this procedure, swine 215 was noted to be ill, with dehydration and constipation, not feeding well. The subject's pre-injury vital signs were poor, with a low MAP, a low heart rate, very low $ETCO_2$, and hypothermia. On top of this, the subject was small (30 kg). Our prediction was that this subject would rapidly succumb to the injury, no matter what the treatment, because of the subject's poor pre-morbid condition. We even had considered not using this subject, because of this debilitated state, but we went ahead anyways.

Surprisingly, the subject survived to 1 h after injury easily, with <1 L blood loss and a good MAP (actually higher than his pre-injury MAP). He only required 500 mL of resuscitation fluid. I'm sure the subject could have easily survived the 3 h observation period, but I had to prematurely end the procedure because of other commitments. At autopsy, the standard injury was present, with red clot (no foam) covering the transected vessels.

One could explain today's survival with any combination of the following mitigating factors:

- Subject was small, with a small liver (575 g) and small vessels
- Subject was hypotensive/dehydrated (low venous pressure)
- Subject was bradycardic (likely low cardiac output & low flow)

Nevertheless, subject did survive & clotted off injury. I'm not sure if biologics contributed, since there was no evidence of foam anywhere near the injury (FI immunohistochemistry of injury site clot pending). So far

we have treated five noncompressible injury subjects with foam + biologics. Four have died quickly, and one (today's subject) survived easily. Compare this with our historical 60% one-hour survival for untreated controls in this model.

VII. PLAN

Repeat above experiment with swine no. 216 on Tue May 6th beginning at 8 AM. I would like to try a new technique with the foam injector tip: instead of passing the injector directly through the incision (between towel clips), I would like to pass the injector through a separate stab incision in the lateral abdominal wall. With this technique, we should have less leakage of foam around the injector tube during the injection, since we can make the separate stab incision for the injector tube very tight around the tube.
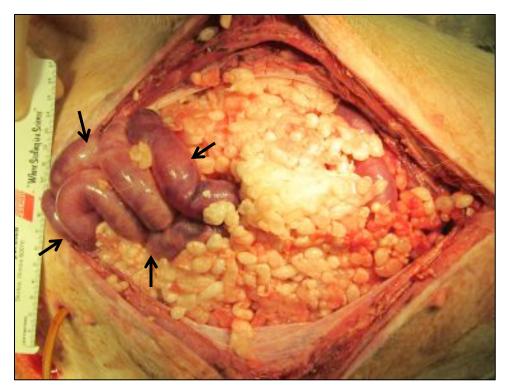
Figure 1, swine 215. Overhead view of the re-opened abdomen 60 min after injury. Subject alive & well. Cephalad to the right. Lobulated foam with minimal blood-staining is visible. Note ischemic-appearing intestine at inferior of wound (arrows).
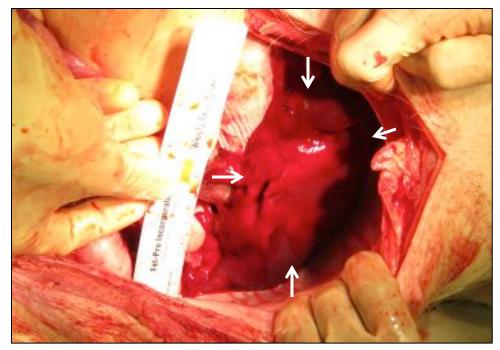
Figure 2, swine 215. Similar view as in Fig. 1. Foam has been evacuated. Subject alive & well. Cephalad to the right. A large red clot (arrows) without foam is visible overlying the liver.
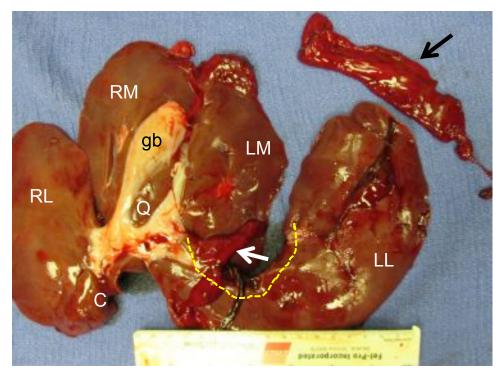
Figure 3, swine 215. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. White arrow = clot overlying injury site. Black arrow = clot formerly stuck to superior surface of liver, now detached. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; gb = gallbladder.
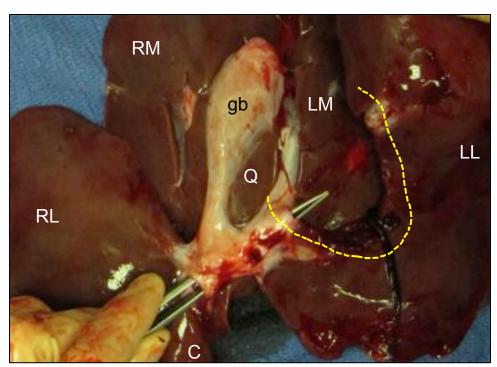
Figure 4, swine 215. Liver *ex vivo,* inferior aspect, showing injury site. Clot in Fig. 3 has been removed. Tips of forceps emerges from cut PV branch to LL lobe. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; gb = gallbladder.
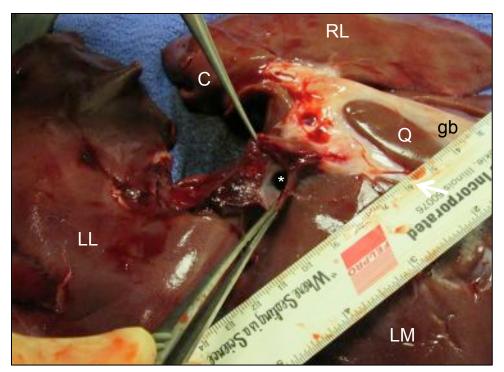
Figure 5, swine 215. Liver *ex vivo,* left inferolateral aspect, showing the base of the injury site. Forceps are distracting open the cut end (*) of the HV to the LL lobe. This end was collapsed and occluded with clot (see Fig. 3). RL = right lateral lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; gb = gallbladder.

I. OVERVIEW
Date: May 6, 2014
Swine no: 216
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Fatemi, Li, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 8:00AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 4/18 /2014
Pre-procedure wt: 47.6kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 150
- MAP: 133
- Temp: 37
- EtCO2: 35

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:32 AM
Spleen wt: 431.3gm
LR (22°C) infused after splenectomy: 1293.9 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 431.3+ 30 + 51.4 = 512.7 mL
- LR (22°C) infused (spleen replacement + incidental): 1293.9 + 300 = 1593.9 mL

Pre-injury VS
- HR: 114
- MAP: 123
- Temp: 36.4

- EtCO2: 33
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:54 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through a separate stab incision in the left lateral side of the abdomen (see Figs) to reduce air leak, with the tip directed into the right paracolic gutter.
Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).
Clotting factors: pdFI (~750 mg total), rFII (Recothrom), rFXIII.
Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam +FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted up & down the right paracolic gutter.
Total mass injected: 240 mL $CaCl_2$, 746.6 g alginate.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR
Time resuscitation fluid began: 08:54 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:05 AM

15 min post-injury VS
- HR: 159
- MAP: 43
- Temp: 36.1
- EtCO2: 24
- IAP: 26

Blood draw no. 3: (30 min post-injury): 09:24 AM

60 min VS
- HR: 141
- MAP: 41
- Temp: 34.1
- EtCO2: 16
- IAP: 34

Survival at 60 min? Yes
Target MAP attained? No
Time of death: 10:22 AM
Cause of death: exsanguination from injury
Interval from injury to death: 88 min

Post-treatment fluid data:
- Blood loss 3632.6 mL (suction) + 1128.3 mL (clot) + 243.7 mL (lap pads) = 5004.6 mL
- IV fluid given: LR (37°C): 7100 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~35 mm Hg). Marked subcutaneous emphysema in both groins; mottled skin (low perfusion state) over the abdomen & groins (see Figs). Upon re-opening abdomen, foam covering the viscera (see Figs). Foam had more of an amorphous configuration today, not so much the peanut-shape configuration we have been seeing recently. The foam was not mixed with blood; the blood tinge was mostly superficial on the foam surface (see Figs). Deep to this layer of foam was a large amount of clotted and unclotted blood. No obvious intestinal ischemia noted, but intestine quite pale.

Volume foam recovered: 827 gms (see Figs).
Heart: No clots or foam present in RV or RA.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe and incomplete transaction of 2nd branch to LL lobe.
Other: none
Ex vivo total liver wt: 932.9 g

Tissue harvested: none

VI. COMMENTS

Relatively large subject today (~48 kg). Survived out to ~1.5 h, died of exsanguination (5 L blood loss). No clotting at injury site. New technique of foam injection tried today with insertion of injector through a separate stab incision (see Figs). This produced a "cleaner" injection with less leakage of foam around the injector. I personally liked this injection technique, and will plan to use it on subsequent pigs. Not sure if this separate-stab incision technique produced any difference in hemostatic efficacy. Today's subject did survive past one hour, but was hypotensive throughout; I would not describe a 5 L blood loss as a "success."

We now have treated six subjects with alginate foam + biologics: four died quickly, one subject (last week's) survived to one hour easily, and today's subject yielded an intermediate result. Not exactly a ringing endorsement of this therapy… but we need more exploratory data. We are not at a point yet where we can run a formal trial/ comparison of different treatments, because I don't think we have a therapy that has reliable efficacy.

One area of the alginate formulation that could be improved is the volume of free butane gas that gets injected with the alginate/FS foam. In this respect I think the volume of "free" gas should be as minimal as possible to maximize the "solid" volume of the alginate/FS foam. We still have to perform a lot of venting through the midline incision to void the abdomen of this free gas.

Regarding the poor mixing of the alginate/FS foam with the endogenous blood, I'm not sure what to do about this, or if it even represents a problem. But given the mediocre/poor performance of the alginate/FS foam to date, we probably should consider the poor mixing as a potential problem.

VII. PLAN

Repeat today's procedure in swine no. 217, on Tue May 13th at 8 AM (single subject again with a 3 h end point).
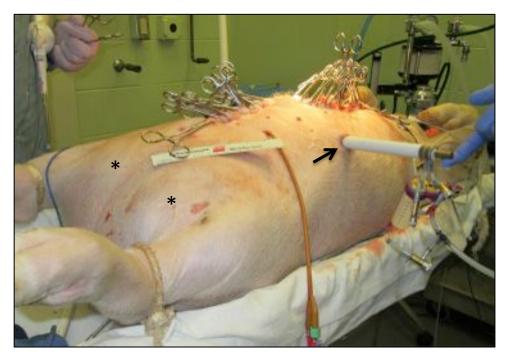
Figure 1, swine 216. View of left side of subject, looking from feet up to head, shortly after completion of the foam injection (time is ~5 min after injury). Midline incision has been closed with towel clips. Note placement of injector (arrow) through a separate stab incision in the left lateral abdominal wall. The bilateral groin in this subject became markedly distended with subcutaneous emphysema (*), caused by the injection. IAP at this point in time ~40 mm Hg.
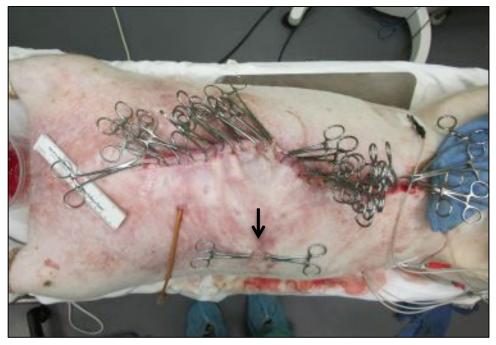
Figure 2, swine 216. Overhead view of abdomen shortly prior to expiration; time is ~75 min after injury. MAP was 23 at this point, and IAP = 31. Anterior surface of abdomen and groins are diffusely mottled (sign of poor perfusion). Midline incision still closed with towel clips. Site of injector placement (arrow) also has been closed with clips. Cephalad is to the right.
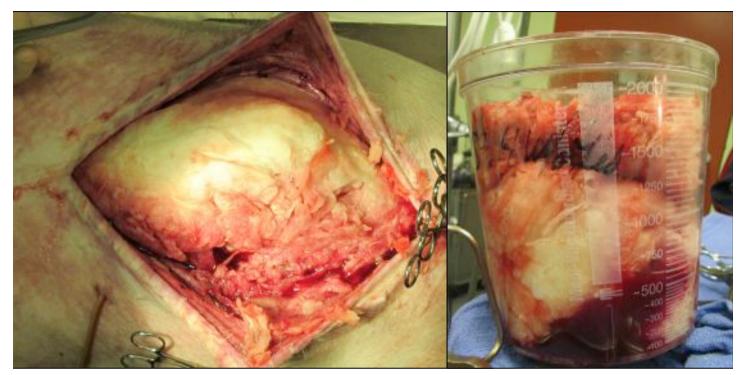
Figure 3, swine 216. Left: overhead view of the re-opened abdomen after subject expired from exsanguination, ~90 min after injury. Morphology of foam was more amorphous compared to recent subjects. Cephalad is to the right. Right: appearance of foam after evacuation into a plastic bucket. Foam was not mixing well with blood & clots.
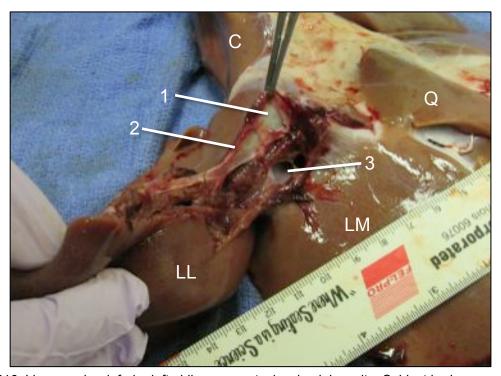
Figure 4, swine 216. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Subject had one completely transected portal vein branch to LL lobe (1), a second PV branch with partial transection (2), and a transected hepatic vein to the LL lobe (3). There was no clot adherent to the injury site. LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe.
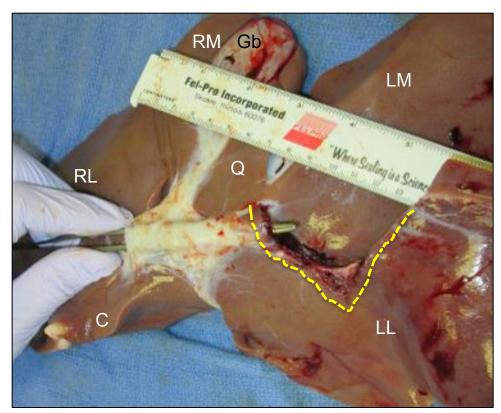
Figure 5, swine 216. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. There was no clot adherent to the injury site. Tips of forceps emerge from transected portal vein branch to LL lobe. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder.

I. OVERVIEW
Date: May 13, 2014
Swine no: 217
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII)
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Fatemi, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 08:10 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 5/9/2014
Pre-procedure wt: 36.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 80
- MAP: 113
- Temp: 38
- EtCO2: 40

Blood draw no. 1 (initial): 08:35 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:55 AM
Spleen wt: 351.3 gm
LR (22°C) infused after splenectomy: 1054 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 351.3+ 30 + 27.9 = 409.2 mL
- LR (22°C) infused (spleen replacement + incidental): 1054 + 25 = 1079 mL

Pre-injury VS
- HR: 97
- MAP: 102
- Temp: 36.5

- EtCO2: 42
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:13 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through a separate stab incision in the left lateral side of the abdomen (see Figs) to reduce air leak, with the tip directed into the right paracolic gutter.

Treatment formulation: calcium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors); ~50% reduction in mass of butane compared to previous subject.

Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.

Technique: (see Figs) with the lower half of the incision closed with towel clips and the injector tubing in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted up & down the right paracolic gutter. Note: IAP rose to ~65 mm Hg initially during injection, then decreased over next 10 min back in the 30-range. Also, only ~50% of intended fibrinogen dose injected today 2° clogged tubing.

Total mass injected: 490.9 gm of alginate, 375 mg of Fibrinogen, 30 mg of FXIII, 9,000 units of Thrombin.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR (5.5 L preset maximum, or 150 mL/kg)

Time resuscitation fluid began: 9:13 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 9:23 AM

15 min post-injury VS
- HR: 144
- MAP: 52
- Temp: 36.6
- EtCO2: 30
- IAP: 32

Blood draw no. 3: (30 min post-injury): 9:43 AM

Final (50 min) VS
- HR: 79
- MAP: 10
- Temp: 34.8
- EtCO2: 0

- IAP: 30

Survival at 60 min? No
Target MAP attained? No
Time of death: 10:03 AM
Cause of death: exsanguination from injury
Interval from injury to death: 50 min

Post-treatment fluid data:
- Blood loss 3026.5 mL (suction) + 917.6 mL (clot) + 0 mL (lap pads) = 3944.1 mL
- IV fluid given: LR (37°C): 5400 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: Abdomen distended, tense (IAP ~30 mm Hg). Upon re-opening abdomen, no free gas present. There was white foam covering the viscera, not mixed with blood (see Figures). Foam had an amorphous configuration. No mixing of foam with blood, except for the staining at the foam-blood interface in the deeper regions. Large amount clotted & unclotted blood recovered. No obvious intestinal ischemia noted and intestines look pale. Some clot was seen near the injury site, but none covering the actual injury (see Figures).
Volume foam recovered: 554.8 g (see Figs)
Heart: A medium sized clot was seen in the right ventricle and a stringy clot was seen extending in the right atrium extending into the inferior vena cava (see Figs), IVC immediately superior to diaphragm was free of clots.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 821.1 g

Tissue harvested: (1) red clot from deep abdomen, infrahepatic space; (2) clot from right ventricle; (3) clot from right atrium.

VI. COMMENTS

Subject dead at 50 min, had red clot cardiac emboli, but 4 L blood loss. I would have to say that death was primarily due to exsanguination. The subject's blood had the appearance of cherry Kool-Aid at the end, i.e., a very low hematocrit not compatible with survival. I don't think the clot emboli represented foam, as there was no evidence of foam anywhere near the injury. Basically what we saw in this subject, and all previous subjects, was a layering of foam over the top of the viscera, with liters of blood and clot underneath. Specifically, no foam gets near the injury site. This is good in that it means that the foam is an unlikely source of cardiac emboli, but perhaps bad for hemostatic efficacy. This last point is relevant in that, aside from one subject (no. 215) that survived easily to one hour, we have had no real evidence of efficacy from our current foam formulation. We did pump up the IAP to 65 mm Hg for a brief period with this subject, and maintained >30 throughout the postinjury period, and also reduced the butane mass (to decrease free gas in the abdomen), but without obvious effect on hemostasis.

VII. PLAN

Repeat in Swine 218 on Tue May 20th at 8 AM.
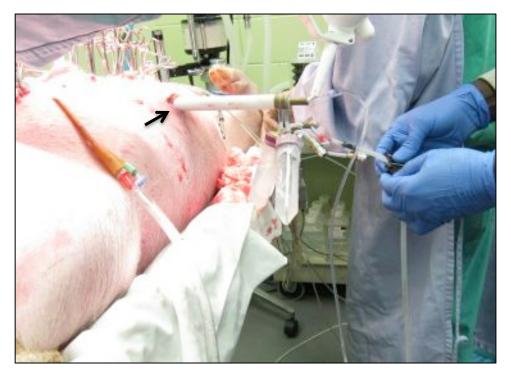
Figure 1, swine 217. View of left side of subject, looking from feet up to head, shortly after completion of the foam injection (time is ~5 min after injury). Midline incision has been closed with towel clips. Note placement of injector (arrow) through a separate stab incision in the left lateral abdominal wall. IAP at this point in time ~60 mm Hg.
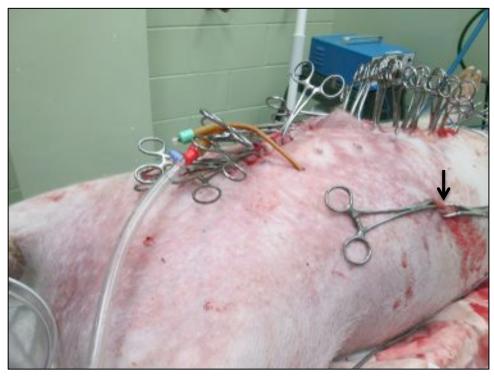
Figure 2, swine 217. Left lateral view of abdomen, looking toward head; time is ~20 min after injury. MAP was 55 at this point, and IAP = 32. Anterior surface of abdomen and groins are diffusely mottled (sign of poor perfusion). Midline incision still closed with towel clips. Site of injector placement (arrow) also has been closed with clips.
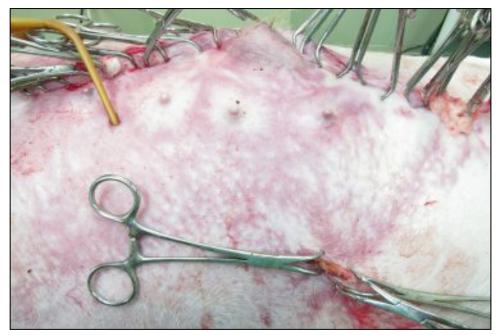
Figure 3, swine 217. Close-up view of abdomen from Figure 2.
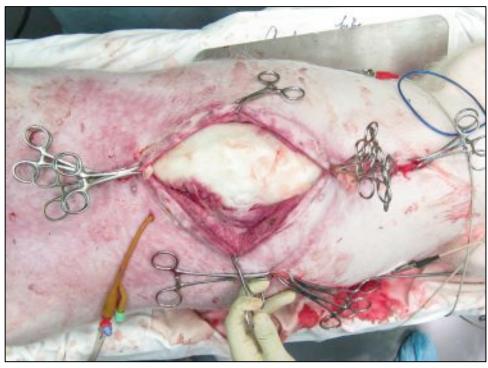
Figure 4, swine 217. Overhead view of partially re-opened abdomen after subject expired from exsanguination 50 min after injury. Morphology of foam was amorphous. Cephalad is to the right. Incision was split apart from intraabdominal pressure as clips were removed.

Figure 5, swine 217. Same view of Figure 4, will all clips removed. Foam still *in situ*. Note minimal mixing of foam with blood. Volume of foam removed ~1.5 L.
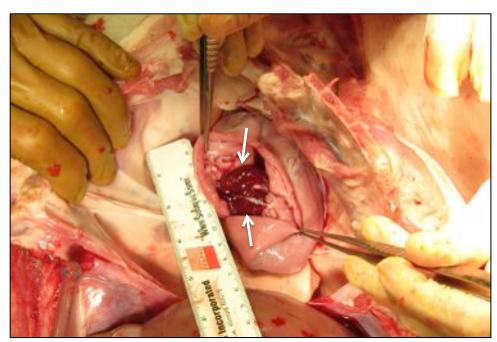
Figure 6, swine 217. View of opened right ventricle *in situ*, which had red clot present inside (arrows). Forceps are distracting cut edges of ventricular walls. No foam or gas bubbles present.
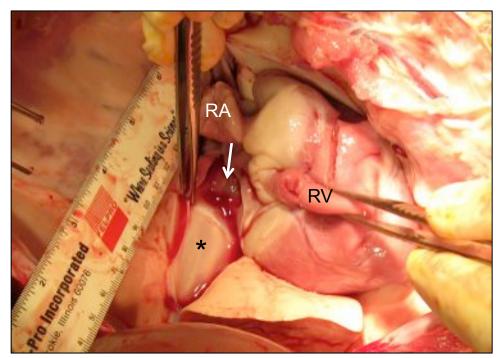
Figure 7, swine 217. View of opened supradiaphragmatic inferior vena cava *in situ*. There was a red clot at the junction of the IVC and the right atrium. RV = right ventricle; RA = right atrium; * = interior of IVC.
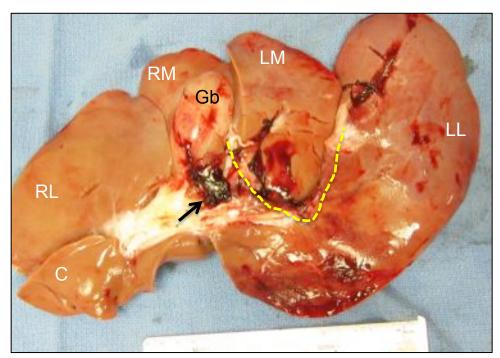
Figure 8, swine 217. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. There was no clot nearby (arrow), but none adherent to the injury site. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Gb = gallbladder.
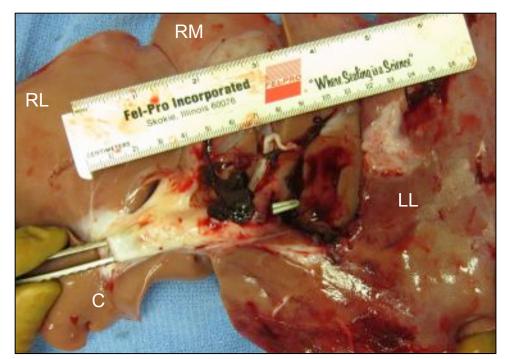
Figure 9, swine 217. Liver *ex vivo,* similar view as in Figure 8. Tips of forceps emerge from transected portal vein branch to LL lobe. RL = right lateral lobe; RM = right medial lobe; LL = left lateral lobe; C = caudate lobe.
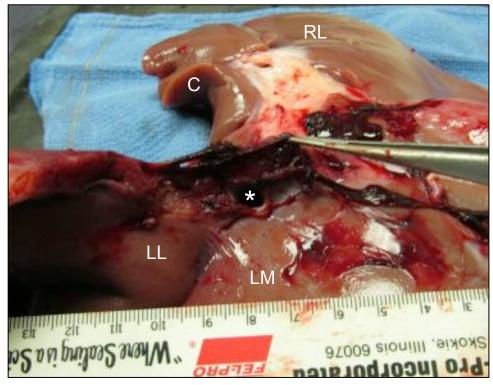
Figure 10, swine 217. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Subject had one completely transected portal vein branch to LL lobe and a transected hepatic vein to the LL lobe (*). There was no clot adherent to the injury site. RL = right lateral lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe.

I. OVERVIEW
Date: May 20, 2014
Swine no: 218
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: calcium alginate foam + fibrin sealant (pdFI, rFII, rFXIII); hypotensive resuscitation protocol
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Fatemi, Ismail, Vanderslice

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II. PRE-INJURY PHASE

Start time: 08:37 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 05/9 /2014
Pre-procedure wt: 39.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot; multiple
    shots required before subject became somnolent
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
    1. Endotracheal tube with ETCO2 monitor
    2. EKG clips
    3. Left ear vein angiocath (20g) for supplemental LR
    4. Right carotid artery angiocath (20g), cutdown; for BP monitor
    5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
    6. Transabdominal cystotomy for 16 Fr Foley catheter
    7. Rectal temp probe
    8. Pulse oximetry
    9. Heating pad below subject
    10. Intraabdominal pressure monitor

Initial VS
    • HR: 86
    • MAP: 118
    • Temp: 37.3
    • EtCO2: 27

Blood draw no. 1 (initial): 08:57 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:15 AM
Spleen wt: 338.0 gm
LR (22°C) infused after splenectomy: 1000 mL at 150 mL/min

Pre-injury fluid data:
    • Blood loss (spleen weight + phlebotomies + incidental): 338+ 0 + 30 = 368 mL
    • LR (22°C) infused (spleen replacement + incidental): 1000+ 200 = 1200 mL

Pre-injury VS
- HR: 96
- MAP: 111
- Temp: 36.2
- EtCO2: 30
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:38 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied across the base of the LLL, in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through a separate stab incision in the left lateral side of the abdomen to reduce air leak, with the tip directed into the right paracolic gutter.

Treatment formulation: calcium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 84 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: pdFI (250 mg total), rFII (Recothrom), rFXIII.

Technique: with the lower half of the incision closed with towel clips and the injector tip in position, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted up & down the right paracolic gutter, including over the top of the liver. IAP did not rise above 20 mm Hg.

Total mass injected: 260 mL $CaCl_2$, 739.4 g alginate.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80 (not used 2° hypotensive resuscitation protocol)

Resuscitation fluid: warm LR, 4.0 L preset maximum (100 mL/kg), given at constant rate of 22 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in <u>hypotensive resuscitation protocol</u> = (Subject wt in kg) x (100/mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:38 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:48 AM

15 min post-injury VS
- HR: 78
- MAP: 111
- Temp: 36.2
- EtCO2: 30
- IAP: 11

Blood draw no. 3: (60 min post-injury): 10:38 AM

Final (180 min) VS
- HR: 68
- MAP: 47
- Temp: 35.7
- EtCO2: 36
- IAP: 13

Survival at 180 min? Yes
Target MAP attained? No
Time of death:12:38 PM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss mL 1151.9 (suction) + 646.1 mL (clot + lap pads) = 1798 mL
- IV fluid given: LR (37°C): 4000 mL @ 22mL/min.

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, modestly firm (IAP ~13 mm Hg). Upon re-opening abdomen, foam covering the viscera (see Figs) in superficial position; not in deep recesses. Small amount of foam above liver. Foam had an amorphous configuration and not mixed with blood (see Figs). Moderate amount clotted & unclotted blood. There was discoloration of the stomach and intestines (see Figs). Clot was adjacent to (possibly had been covering) injury site (see Figs).
Volume foam recovered: 2+ L
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe (see Figs)
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 1022.2 g

Tissue harvested: two clots: (1) from upper abdomen/infrahepatic space, freely floating, appeared to contain foam; (2) red clot adherent to liver, adjacent to injury site.

VI. COMMENTS

Very interesting result today with subject 208. This is the first three-hour survival result we have obtained. Blood loss was 1.7 L. The main thing that was done differently today was a "hypotensive resuscitation" protocol; everything else about today's procedure was essentially unchanged.

The concept of hypotensive resuscitation has been around for about about a century, and basically says that subjects (both animal and human) with uncontrolled hemorrhage will do better if crystalloid resuscitation fluids are restricted in the pre-hospital phase (i.e., in the time period prior to when the subject can have operative control of the hemorrhage site.

The definition of "hypotensive resuscitation" is variable from paper to paper. Some folks mean absolutely no IVF should be given. The Army recommends hypotensive resuscitation for injured combatants with uncontrolled hemorrhage in the pre-hospital phase, who are not mentating and/or do not have a palpable radial

pulse. In these patients, a maximum of 1 L Hextend (6% hetastarch) may be given prior to arrival at a surgical unit.

Although we have been restricting our administration of post-injury resuscitation LR to 100 mL/kg, we have been giving this at 150 mL/min, which means that the fluid resuscitation is completed within the first half hour after injury; i.e., basically as a massive bolus. Today, we used the same total volume restriction (100 mL/kg, or ~4 L in today's subject), but spread the administration over the entire 180 min (which calculates to an IVF rate of 22 mL/min, or 15% of the previous rate).

The fact that the subject can do better with a lower IVF rate seems counter-intuitive, but that is exactly what we observed today. Obviously we only have N = 1 with this different protocol, but the results are exciting.

I tried this protocol today because our treatment has been showing little if any efficacy… so, basically, I was desperate to try something different. I guess the reason why we haven't used a more restricted fluid resuscitation protocol before this was that, with a couple of exceptions, none of the other contemporary groups investigating hemorrhage in porcine models have utilized severe fluid restrictions.

Important caveat: with the use of a hypotensive resuscitation protocol, all of the no-treatment controls will have to be redone. Obviously, if the no-treatment controls also survive out to 180 min, then we'll need a new model.

Unfortunately, issue of calcium-induced intestinal injury is not going away (see Figures). I don't have a good answer for this at this point… but we will have to address this at some point. No matter how efficacious a treatment might be, it won't get through the FDA process with this kind of toxicity.

Heart not examined today. I have noted that when we transect the supradiaphragmatic IVC for euthanasia, the open end of the IVC on the cardiac side can suck in air or clot or whatever floats up in the chest cavity. The diaphragm is opened to perform euthanasia, so all the material in the abdomen can get into the chest during this period. So perhaps emboli we have seen in the past got into the heart via this mechanism, which means that the were not true emboli. In the future, when we want to examine the heart for emboli, we will clamp the IVC just inferior to the heart prior to transecting the supradiaphragmatic IVC. This will prevent false-positive emboli from entering the RA or RV.

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VII. PLAN

No procedures next week; next week we will present data to ISR in San Antonio. Next swine procedure will be in two weeks, on Tue June 3, 2014 at 8 AM. We will repeat today's experiment, sticking with a hypotensive resuscitation protocol, as described on p. 2.

Swine 218/ Page 4 of 4
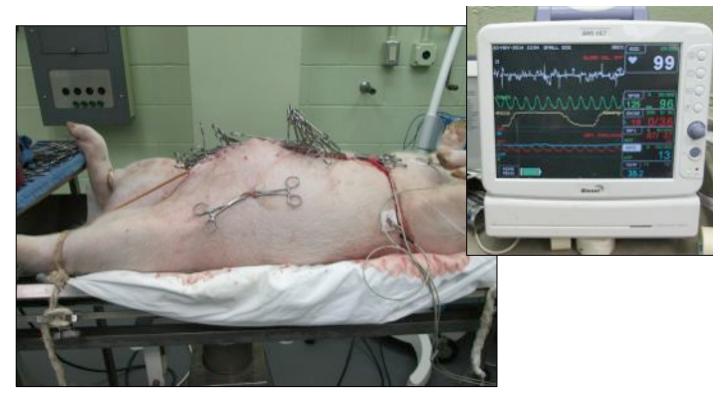
Figure 1, swine 218. View of left side of subject, cephalad is to right. Time = 2 h, 55 min after injury. Swine alive with MAP 46 mm Hg, IAP = 13 mm Hg, EtPCO2 = 36 mm Hg, temp = 35.2˚C (inset, monitor view). Total IVF given post-injury ~4 L, at 22 mL/min.

Figure 2, swine 218. Overhead view of re-opened abdomen 3 h after injury; subject still alive with MAP ~40. Foam morphology = amorphous. Foam completely covering intestines; minimal mixing of foam with blood. Cephalad is to the right.
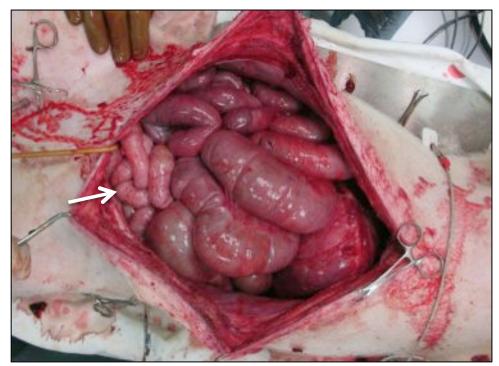
Figure 3, swine 218. Same view as Fig. 2, immediately after foam evacuation. Subject still alive. Note diffusely ischemic appearing intestines; compare with normal appearing small intestine (white arrow).
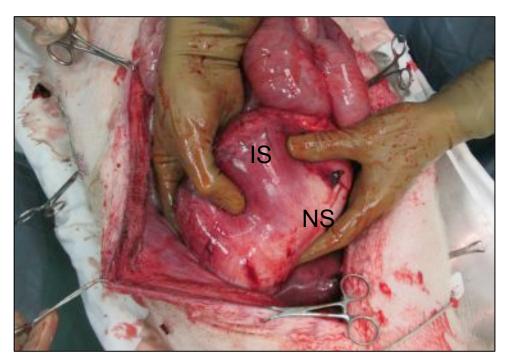
Figure 4, swine 218. Overhead view, immediately after foam evacuation. Subject still alive. Anterior surface of stomach displayed by operator's hands, showing demarcation between ischemic-appearing stomach (IS) and normal-appearing stomach NS). Cephalad toward bottom of image.
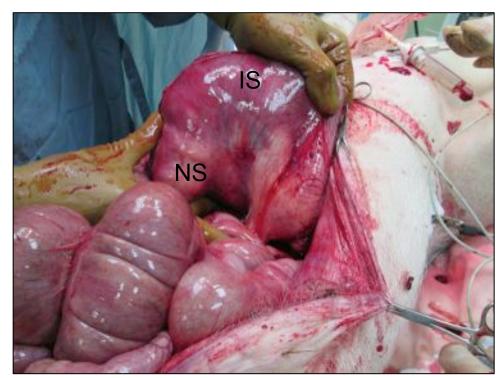
Figure 5, swine 218. View toward head, immediately after foam evacuation. Subject still alive. Posterior surface of stomach displayed by operator's hands, showing demarcation between ischemic-appearing stomach (IS) and normal-appearing stomach NS).
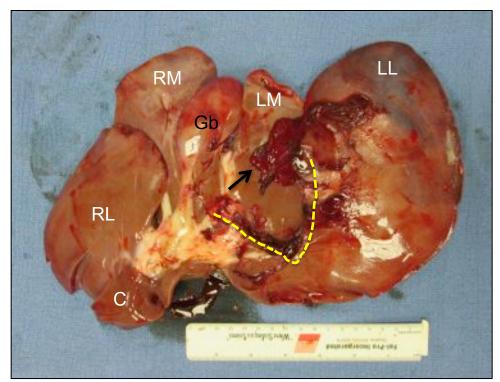

Figure 6, swine 218. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. Large clot was adjacent to injury site (arrow). RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Gb = gallbladder.
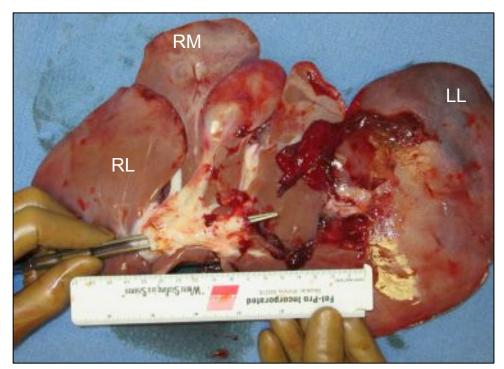
Figure 7, swine 218. Liver *ex vivo,* similar view as in Figure 8. Tips of forceps emerge from transected portal vein branch to LL lobe. RL = right lateral lobe; RM = right medial lobe; LL = left lateral lobe.
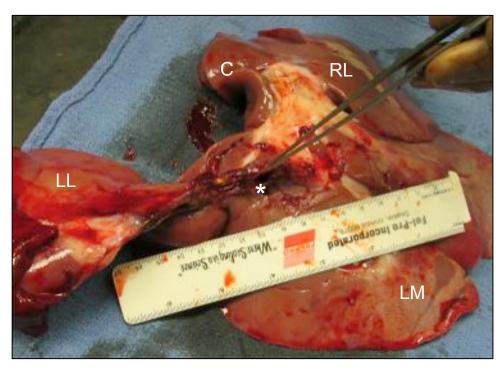
Figure 8, swine 218. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Subject had one completely transected portal vein branch to LL lobe and a transected hepatic vein to the LL lobe (*). RL = right lateral lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe.

Figure 9, swine 218. Close-up of clot visualized in Fig. 6 & 7. Clot appearance is somewhat homogeneous, suggesting something more than endogenous clot.

I. OVERVIEW
Date: June 3, 2014
Swine no: 219
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: none (no treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Siford.

II. PRE-INJURY PHASE

Start time: 7:55 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 5/9/2014
Pre-procedure wt: 48.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 130
- MAP: 117
- Temp: 38.8
- EtCO2: 36

Blood draw no. 1 (initial): 08:22 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:37 AM
Spleen wt: 369.9 gm
LR (22°C) infused after splenectomy: 1100 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 369.9+ 20 + 38.2 = 428.1 mL
- LR (22°C) infused (spleen replacement + incidental): 1110 + 50 = 1160 mL

Pre-injury VS
- HR: 82
- MAP: 117
- Temp: 38.1

- EtCO2: 36
- IAP: 0

III. INJURY & OBSERVATION PHASE

Time of injury: 08:49 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision.

Treatment formulation: No treatment.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 4.8 L preset maximum (100 mL/kg), given at constant rate of 27 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in <u>hypotensive resuscitation protocol</u> = (Subject wt in kg) x (100/mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:50 AM (within 1 min of injury)

IV. POST- INJURY PHASE

Blood draw no. 2 (10 min post-injury): 09:00 AM

15 min post-injury VS
- HR: 71
- MAP: 63
- Temp: 37.4
- EtCO2: 30
- IAP: -3

Blood draw no. 3: (30 min post-injury): 09:30 AM

Final (180 min) VS
- HR: 112
- MAP: 97
- Temp: 35.6
- EtCO2: 33
- IAP: -2

Survival at 180 min? Yes
Target MAP attained? Briefly/intermittently
Time of death: 11:49 AM
Cause of death: exsanguination from euthanasia

Interval from injury to death: 180 min

Post-injury fluid data:
- Blood loss: 1136 mL (lap pads only, no suction)
- IV fluid given: LR (37°C): 5050 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft (IAP ~0 mm Hg). Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A small clot was covering injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 978 g

Tissue harvested: none

VI. COMMENTS

No treatment control of noncompressible injury model, using restricted fluid administration rate (100 mL/kg spread out evenly throughout the 180 min observation period; 27 mL/min for this subject). Subject survived quite easily with ~1 L blood loss. Was at or near target MAP during the final hour.

I would guess that giving the entire 100 mL/kg of resuscitation fluid during the 1st 30 min, which is what we have been doing for the past year, has been diluting out the endogenous clotting factors, and causing premature deaths in what otherwise would have been a survivable injury. Of course, this represents only N = 1 for the no treatment control group with restricted fluid, but the difference from previous controls is remarkable.

If the present injury mechanism (hemitransection of the left lateral lobe at its base) turns out not to be fatal with restricted fluid administration, then the noncompressible model will need to be revised.

VII. PLAN

Repeat this sequence on Tue June 10th to ensure that it was not a fluke.

Figure 1, swine 219. View of left side of subject, cephalad is to right. Time = 2 h, 25min after injury. Swine alive with MAP 92mm Hg, IAP = -2 mm Hg, EtPCO2 ~30 mm Hg, temp = 35.8˚C (inset, monitor view). Total IVF given post-injury 100 mL/kg, @ 27 mL/min.
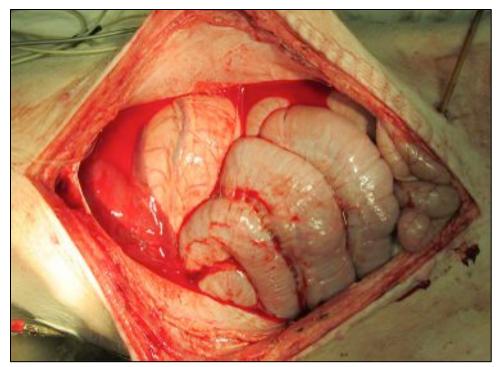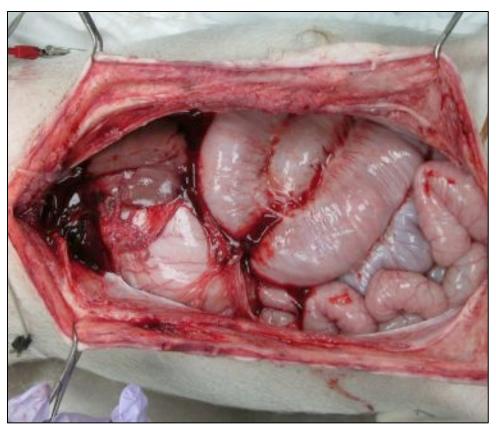

Figure 2, swine 219. Overhead view of re-opened abdomen 3 h after injury; subject still alive with MAP ~70. Modest amount of free blood in abdominal cavity. Cephalad is to the left.
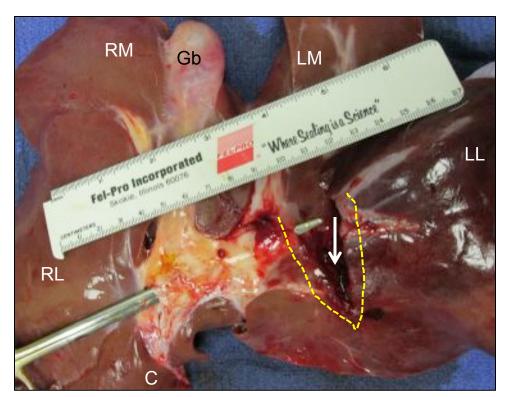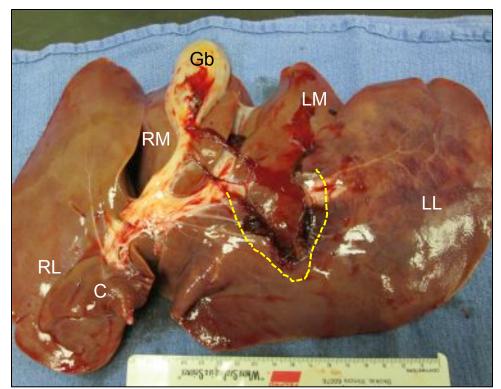
Figure 3, swine 219. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut; this was manually reapproximated postmortem compared to images of previous subjects. Clot was adherent to injury site (white arrow). RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Gb = gallbladder. Tips of scissors emerge from transected portal vein branch (1 of 2) to LL lobe.
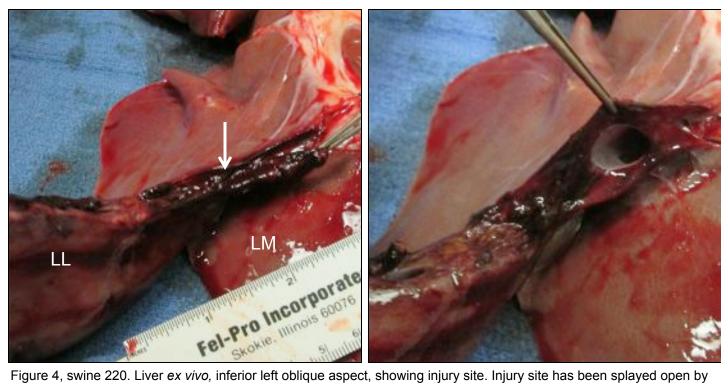

Figure 4, swine 219. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Injury site has been splayed open by distracting LL lobe laterally. White arrow indicates clot obliterating orifice of transected hepatic vein from LL lobe.
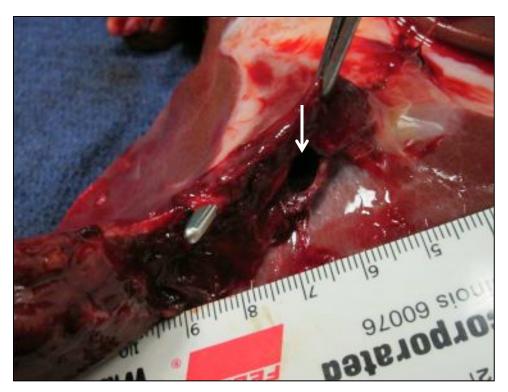
Figure 5, swine 219. Liver *ex vivo,* similar view as in Fig. 4. Close of up injury site; hilum of liver is toward top of image. Tips of scissors shown emerging from transect branch (2 of 2) of portal vein to LL lobe; other transected PV branch shown in Fig. 3. The clot covering the HV orifice in Fig. 4 has been removed, exposing the orifice of the transected HV (white arrow).

I. OVERVIEW
Date: June 10, 2014
Swine no: 220
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford

II. PRE-INJURY PHASE

Start time: 08:03 AM
Swine sex: male (barrow)
Date swine received from UNL Mead: 05/9/2014
Pre-procedure wt: 47.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
• HR: 108
• MAP: 138
• Temp: 38.2
• EtCO2: 39

Blood draw no. 1 (initial): 8:28 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:39 AM
Spleen wt: 321.4 gm
LR (22°C) infused after splenectomy: 1000 mL at 150 mL/min

Pre-injury fluid data:
• Blood loss (spleen weight + phlebotomies + incidental): 321.4 + 20 + 12.8 = 354.2 mL
• LR (22°C) infused (spleen replacement + incidental): 1000 + 50 = 1050 mL

Pre-injury VS
• HR: 100
• MAP: 134
• Temp: 37.2

- EtCO2: 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:54 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision.

Treatment formulation: No treatment.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 100

Resuscitation fluid: warm LR, 4.8 L preset maximum (100 mL/kg), given at constant rate of 26 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:55 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:04 AM

10 min post-injury VS
- HR: 126
- MAP: 39
- Temp: 37.2
- EtCO2: 27
- IAP: 1

Blood draw no. 3: (30 min post-injury): 09:24 AM

30 min VS
- HR: 137
- MAP: 38
- Temp: 37.2
- EtCO2: 30
- IAP: 1

Blood draw no. 4: (60 min post-injury): 09:54 AM

60 min VS
- HR:127

- MAP:51
- Temp:36.9
- EtCO2:33
- IAP:1

Blood draw no. 5: (90 min post-injury): 10:24 AM

90 min VS
- HR:134
- MAP:57
- Temp:36.7
- EtCO2:35
- IAP:2

Blood draw no. 6: (120 min post-injury): 10:54 AM

120 min VS
- HR:121
- MAP:63
- Temp:36.5
- EtCO2:33
- IAP:1

Blood draw no. 7: (150 min post-injury): 11:24 AM

150 min VS
- HR:117
- MAP:73
- Temp:36.3
- EtCO2:32
- IAP:1

Blood draw no. 8: (180 min post-injury): 11:54 AM

180 min VS
- HR:117
- MAP:72
- Temp:36.2
- EtCO2:30
- IAP:1

Survival at 180 min? Yes
Target MAP attained? No
Time of death: 11:54 PM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 237.5 mL (suction) + 1169.3 mL (clot + lap pads) = 1407 mL
- IV fluid given: LR (37°C): 5090 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft (IAP ~2 mm Hg); see Figures. Upon re-opening abdomen, mostly established clot in the superficial & superior regions (see Figs). Upon further exploration, fresh bleeding seen, but this presumably was caused by the exploration. A small clot was covering injury site, seen at *ex vivo* examinatioin. (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 1225.3 g
Tissue harvested: none

VI. COMMENTS

Subject survived easily to 180 min, though with lower MAP than seen last week. We now have N = 2 with noncompressible injury/no treatment/restricted IV fluid rate, both surviving to 180 min. I think N = 4 of 180 min no-treatment survivors would be enough to demonstrate that current model will not be "fatal" enough for a noncompressible model, using the new IV restriction.

VII. PLAN

Repeat the above in two more subjects. Next subject will either be Wed June 18th or Wed June 25th, depending on subject availability.
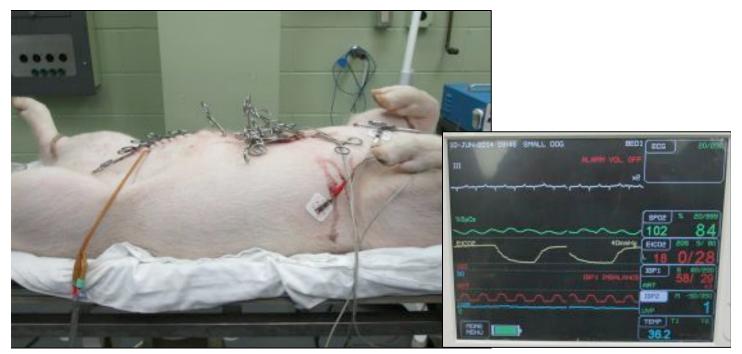
Figure 1, swine 220. View of left side of subject, cephalad is to right. Time = 180 min after injury. Swine alive with MAP 43 mm Hg, IAP = 1 mm Hg, ETpCO2 ~28 mm Hg, temp = 36.2˚C (inset, monitor view). Total IVF given post-injury 100 mL/kg, @ 26 mL/min.

Figure 2, swine 220. Overhead view of re-opened abdomen 3 h after injury; subject still alive with MAP ~40. Very little free blood in abdominal cavity; mostly clot. Cephalad is to the left.

Figure 3, swine 220. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Gb = gallbladder.
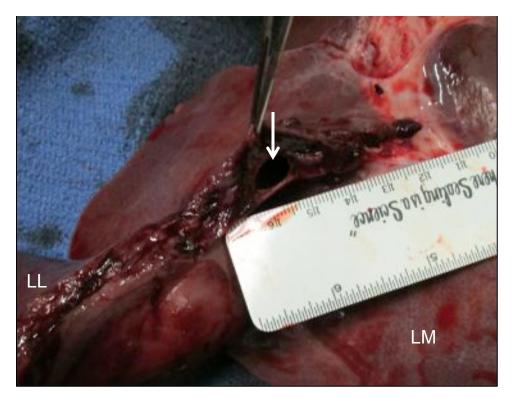

Figure 4, swine 220. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Injury site has been splayed open by distracting LL lobe laterally. White arrow in left image indicates clot obliterating orifice of transected hepatic vein from LL lobe. Forceps in right image demonstrates orifice of this HV after covering clot was removed.

I. OVERVIEW
Date: June 18, 2014
Swine no: 222
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford, Cavanaugh.

II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: male
Date swine received from UNL Mead: 06/13/2014
Pre-procedure wt: 34.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 115
- MAP: 92
- Temp: 36.8
- EtCO2: 36
- IAP: monitor not placed

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:08 AM
Spleen wt: 291.8 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 291.8 + 20 + 173.9 = 485.7 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 50 = 950 mL

Pre-injury VS
- HR: 70
- MAP: 89

- Temp: 36.5
- EtCO2: 36

III. INJURY & TREATMENT PHASE

Time of injury: 09:17 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None.
Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 80
Resuscitation fluid: warm LR, 3.4 L preset maximum (100 mL/kg), given at constant rate of 19 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 09:19 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:27 AM

10 min post-injury VS
- HR: 101
- MAP: 38
- Temp: 36.1
- EtCO2: 31
- IAP: 0

Blood draw no. 3: (30 min post-injury): 09:47 AM

30 min VS
- HR: 134
- MAP: 16
- Temp: 35.5
- EtCO2: 14
- IAP: 0

Survival at 180 min? No
Target MAP attained? No
Time of death: 09:52 AM
Cause of death: exsanguination from injury
Interval from injury to death: 35 min

Post-treatment fluid data:
 • Blood loss: 921.5 mL (suction) + 684.1 mL (clot + lap pads) = 1605.6 mL
 • IV fluid given: LR (37°C): 700 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate
    amounts of blood and clots were seen (see Figs). A moderate sized clot was covering injury site (see Figs).
Heart: no clots or air emboli; heart contained nonclotted blood, moderately distended. IVC was clamped above
    the diaphragm just after death, prior to the abdominal exploration (in order to prevent post-mortem artifactual
    embolism).
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 903.5 g

Tissue harvested: none

VI. COMMENTS

        In stark contrast to previous two subjects, this subject died relatively early (35 min) after injury. Subject
only lost 1.6 L blood; but there was a ~500 mL pre-injury loss that was incurred during neck line placement
(much higher than normal—we experienced technical difficulties during placement of this subject's neck lines).
Importantly, there was no evidence of cardiac embolism. Interestingly, the heart was somewhat distended with
nonclotted blood. This is interesting because subjects expiring from exsanguination typically have minimally
filled or empty cardiac chambers. So I'm not sure if there was some unspecified cardiac dysfunction
accompanying the exsanguination.
        The problem now is that we have two 180 min survivors, and one 35 min death. So we likely will have to
repeat this protocol a total of ~10 times to find out where the actual trend will be with respect to survival. So goes
research with actual living subjects…
        In anticipation of possibly needing a more severe injury mechanism, I performed a "right hepatic
lobectomy" on the ex vivo liver (see attached Figures). By transecting the liver in half, through the anatomic
plane between the right medial and left medial lobes, the left main portal vein and left-sided hepatic veins are
transected (in addition to the left-sided hepatic arteries). This lobectomy mechanism would result in a more severe
injury than presently used and, if the resected left side of the liver was displaced, the lobectomy mechanism also
could present a parenchymal surface that would be in direct contact with the foam treatment.

VII. PLAN

        Repeat this protocol as above (standard injury = LLL hemistransection, no treatment, IVF restriction) on
Fri June 20th.

Figure 1, swine 222. Overhead view of re-opened abdomen ~40 min after injury; subject expired at 35 min. Moderate amount free blood & clot in abdominal cavity. Cephalad is to the left.
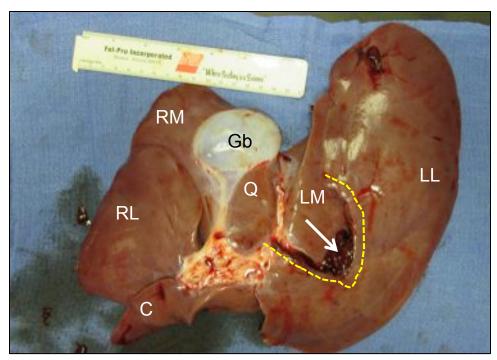
Figure 2, swine 222. Liver *ex vivo,* inferior aspect, showing injury site, which had large clot at the base (arrow). Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder.

Figure 3, swine 222. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Injury site has been splayed open by distracting LL lobe laterally. White arrow in left image indicates orifice of transected hepatic vein from LL lobe, after removal of overlying clot. This orifice leads directly to the intrahepatic IVC.
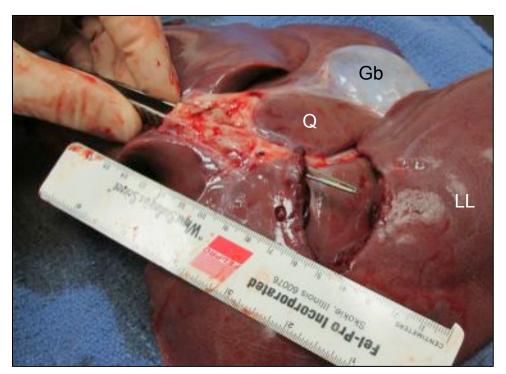
Figure 4, swine 222. Liver *ex vivo,* inferior left oblique aspect, showing injury site. Forceps has been inserted through proximal portal vein, and the forceps tips exit from the transected portal vein branch to the LL lobe.
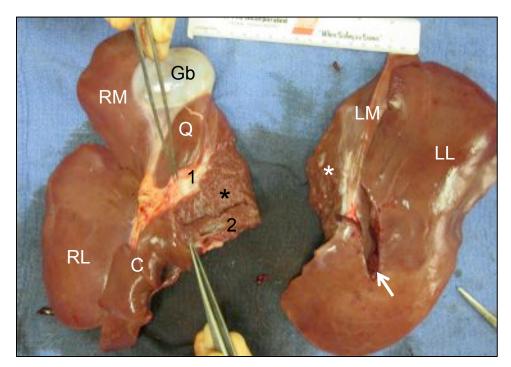
Figure 5, swine 222. Liver *ex vivo,* inferior aspect. A cut has been made in the anatomic plane between the right and left sides of the liver, separating the organ into the two main lobes. The right sided structures include the RL lobe, RM lobe, quadrate lobe, caudate lobe, and gallbladder. The IVC (not visible) runs through the right side of the liver. The left sided structures include the LM and LL lobes. This cut transects the left main branch of the PV (1) and the hepatic veins to both the LM and LL lobes (2; consisting of a common trunk in this specimen). Cut surface of liver indicated with asterisks (*).
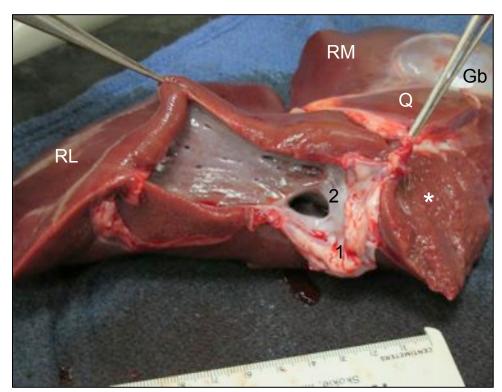
Figure 6, swine 222. Isolated view of right-sides structures from Fig. 5. The posterior wall of the intrahepatic IVC has been cut along its axial axis in order to show the interior of the IVC. 1 = diaphragmatic end of the IVC; 2 = main trunk of hepatic veins (this subject had one primary HV emptying into IVC; HVs to lobes split off this primary HV trunk). Cut surface of liver indicated with asterisk (*). The IVC is close to but not in contact with the anatomic plane separating the right side from the left side.
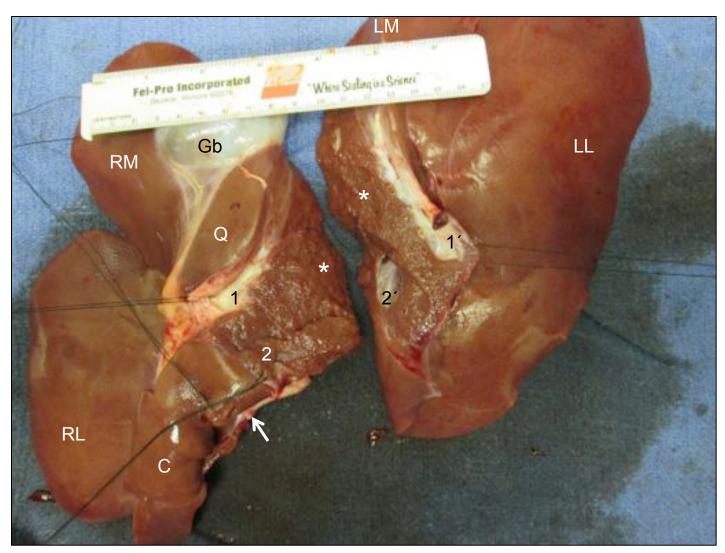
Figure 7, swine 222. Additional view, similar to Fig. 5, with silk stay sutures. A cut was made in the anatomic plane between the right and left sides of the liver, separating the organ into the two main lobes. The right sided structures include the RL lobe, RM lobe, quadrate lobe, caudate lobe, and gallbladder. The IVC (not visible, but indicated with white arrow) runs through the posterior part of the right side of the liver. The left sided structures include the LM and LL lobes. This cut transects the left main branch of the PV (1) and the hepatic veins to both the LM and LL lobes (2; consisting of a common trunk in this specimen). The corresponding ends of the transected PV and HV in the left half of the liver are labeled with 1´ and 2´, respectively. Cut surface of liver indicated with asterisks (*).

I. OVERVIEW
Date: June 20, 2014
Swine no: 223
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male
Date swine received from UNL Mead: 06/13/2014
Pre-procedure wt: 36.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 94
- MAP: 122
- Temp: 36.6
- EtCO2: 39

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:25 AM
Spleen wt: 389.3 gm
LR (22°C) infused after splenectomy: 1170 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 389.3 + 20 + 17.7 = 427 mL
- LR (22°C) infused (spleen replacement + incidental): 1170 + 50 = 1220 mL

Pre-injury VS
- HR: 94
- MAP: 122
- Temp: 36.6
- EtCO2 : 49

III. INJURY & TREATMENT PHASE

Time of injury: 08:54 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 95
Resuscitation fluid: warm LR, 3.6 L preset maximum (100 mL/kg), given at constant rate of 19 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:40AM (within 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:48 AM

10 min post-injury VS
- HR: 89
- MAP: 52
- Temp: 36.3
- EtCO2: 43

Blood draw no. 3: (30 min post-injury): 09:08 AM

30 min VS
- HR: 145
- MAP: 53
- Temp: 35.6
- EtCO2: 43

Blood draw no. 4: (60 min post-injury): 09:38AM

60 min VS
- HR:78
- MAP:59
- Temp:35.3
- EtCO2:41

Blood draw no. 5: (90 min post-injury): 10:08 AM

90 min VS
- HR:77
- MAP:63
- Temp:34.7
- EtCO2:36

Blood draw no. 6: (120 min post-injury): 10:38 AM

120 min VS
- HR:84
- MAP:65
- Temp:34.6
- EtCO2:38

Blood draw no. 7: (150 min post-injury): 11:08 AM

150 min VS
- HR:88
- MAP:70
- Temp:34.4
- EtCO2:36

Blood draw no. 8: (180 min post-injury): 11:38 AM

180 min VS
- HR:90
- MAP:72
- Temp:34.5
- EtCO2:36

Survival at 180 min? Yes
Target MAP attained? No
Time of death: 11:38 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 674.9 mL (suction) + 595.8 mL (clot + lap pads) = 1270.7 mL
- IV fluid given: LR (37°C): 3660 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A moderate size clot was covering injury site (see Figs).
Heart: not examined.

Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 1065.2 g

Tissue harvested: none

VI. COMMENTS

Easy survival to 180 min in this untreated standard injury (LLL hemitransection). So after 4 subjects, we have 3 survivals, and 1 death at 35 min. More subjects needed.
Similar to last subject, post-mortem ex vivo left-sided lobectomy performed to demonstrate anatomy of this injury (see Figs); such an injury should have greater severity than the present mechanism.

VII. PLAN

Next subjects in this series will be on Wed July 2nd at 8 AM, and then on July 10th and 11th (one subject per day).
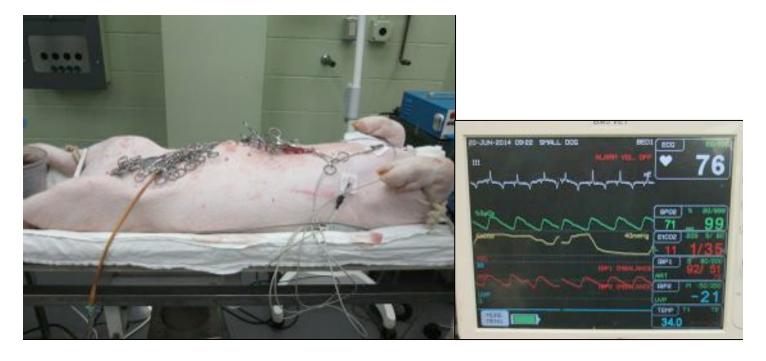
Figure 1, swine 223. Lateral view of abdomen at 160 min after injury. Subject alive & well, with heart rate = 76, O2 sat = 99%, pCO2 = 35, MAP = 73, tem = 34.0. Cephalad is to the right.
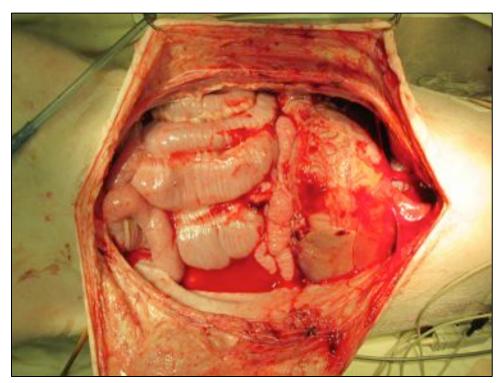
Figure 2, swine 223. Overhead view of re-opened abdomen 180 min after injury; subject subject alive & well. Moderate amount free blood & clot in abdominal cavity. Cephalad is to the right.
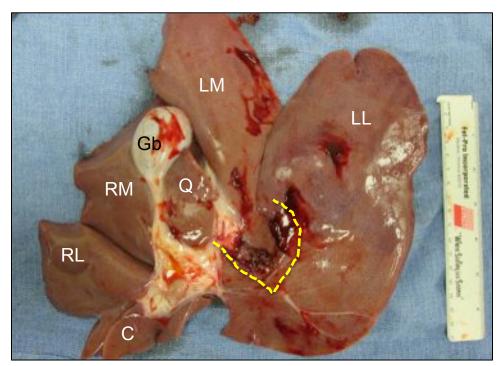
Figures, Swine 223, p. 1 of 2
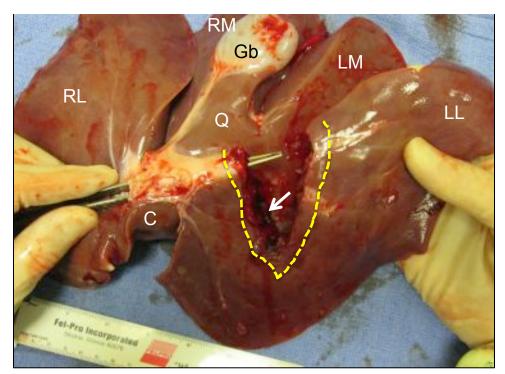
Figure 3, swine 223. Liver *ex vivo,* inferior aspect, showing injury site, which had large clot at the base (arrow). Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder. Forceps tip emerging from cut branch of PV.
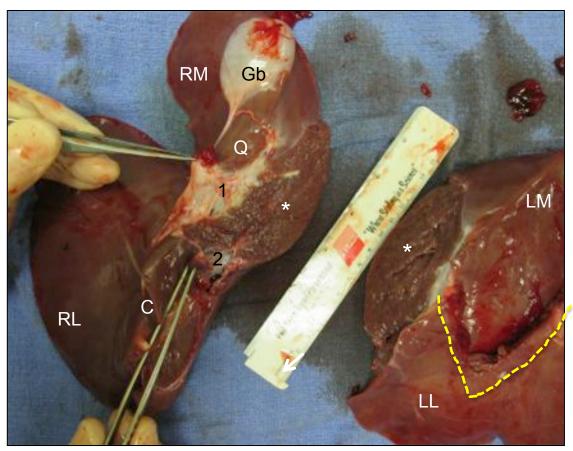

Figure 4, swine 223. A cut was made in the anatomic plane between the right and left sides of the liver, separating the organ into the two main lobes. The right sided structures include the RL lobe, RM lobe, quadrate lobe, caudate lobe, and gallbladder. The IVC (not visible) runs through the posterior part of the right side of the liver. The left sided structures include the LM and LL lobes. This cut transects the left main branch of the PV (1) and the hepatic veins to both the LM and LL lobes (2). Cut surface of liver indicated with asterisks (*).

I. OVERVIEW
Date: July 2, 2014
Swine no: 226
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: male
Date swine received from UNL Mead: 06/26/2014
Pre-procedure wt: 37.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 82
- MAP: 87
- Temp: 37.8
- EtCO2: 33

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:39 AM
Spleen wt: 419.1 gm
LR (22°C) infused after splenectomy: 1260 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 419.1 + 30 + 0 = 449.1 mL
- LR (22°C) infused (spleen replacement + incidental): 1260 + 50 = 1310 mL

Pre-injury VS
- HR: 102
- MAP: 105
- Temp: 36.5

- EtCO2 : 27
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:51 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 85
Resuscitation fluid: warm LR, 3.8 L preset maximum (100 mL/kg), given at constant rate of 21 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:52AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:01 AM

10 min post-injury VS
- HR: 110
- MAP: 18
- Temp: 36.1
- EtCO2: 12
- IAP: 0

Blood draw no. 3: (30 min post-injury): 09:21 AM

30 min VS
- HR: 113
- MAP: 20
- Temp: 35.8
- EtCO2: 12
- IAP: 0

Blood draw no. 4: (60 min post-injury): 09:51AM

60 min VS
- HR:108
- MAP:29
- Temp: 35.1
- EtCO2:22
- IAP:0

Blood draw no. 5: (90 min post-injury): 10:21 AM

90 min VS
- HR: 93
- MAP: 43
- Temp: 34.6
- EtCO2: 24
- IAP: 0

Blood draw no. 6: (120 min post-injury): 10:51 AM

120 min VS
- HR: 104
- MAP: 34
- Temp: 34.4
- EtCO2: 22
- IAP:0

Blood draw no. 7: (150 min post-injury): 11:21 AM

150 min VS
- HR:104
- MAP: 36
- Temp: 34.2
- EtCO2: 21
- IAP:0

Blood draw no. 8: (180 min post-injury): 11:51 AM

180 min VS
- HR: 96
- MAP: 47
- Temp: 33.9
- EtCO2: 21
- IAP: 0

Survival at 180 min? Yes
Target MAP attained? No
Time of death: 11:51 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 1130 mL (suction) + 943.9 mL (clot + lap pads) = 2074 mL
- IV fluid given: LR (37°C): 3780 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). No clot was covering the injury site (see Figs).
Heart: no clots or air in the right ventricle; RV was full of blood. IVC was clamped prior to opening RV.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 947.2 g

Tissue harvested: none

VI. COMMENTS

Hypotensive, 2+ L blood loss, but subject survived easily to 180 min. The tally to date with the restricted IVF resuscitation is: 4 survivals, 1 death.

We will need some sort of follow-up letter/note/short paper to the *PLOS ONE* article, describing what happens to our model when IVF resuscitation is restricted.

I am drawing up plans for a more lethal model. This will need both local IACUC and DoD/ACURO approval prior to implementation.

VII. PLAN

Next subjects for above protocol (untreated LLL hemitransection with IVF restriction) will be July 10 and 11 (Thu & Fri next week).
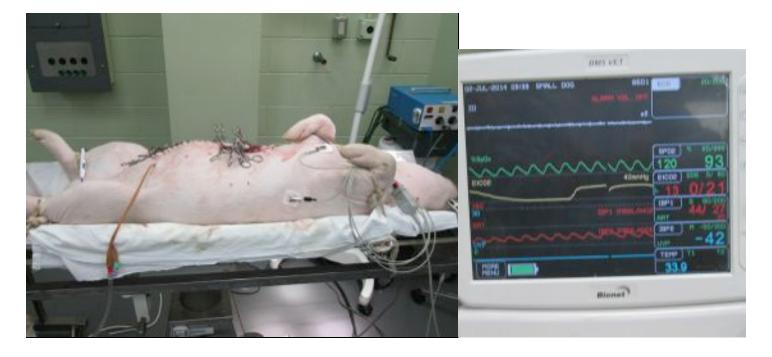
Figure 1, swine 226. Lateral view of abdomen at 175 min after injury. Subject alive, with heart rate = 120, O2 sat = 93%, endtidal pCO2 = 21, MAP = 35, temp = 33.9. Cephalad is to the right.

Figure 2, swine 226. Overhead view of re-opened abdomen 180 min after injury; subject subject alive, MAP ~25. Moderate amount free blood & clot in abdominal cavity. Cephalad is to the left.
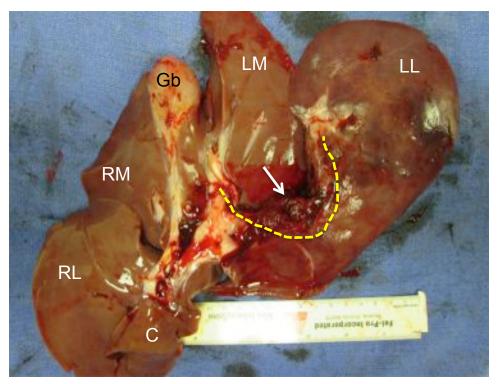
Figure 3, swine 226. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder.
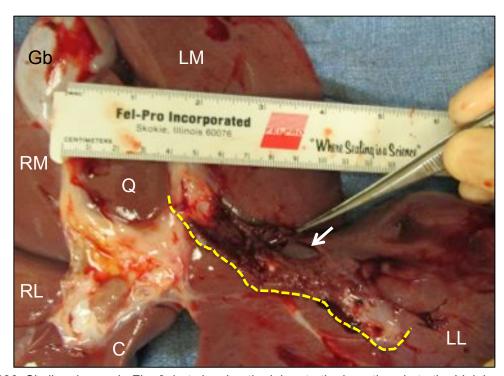
Figure 4, swine 226. Similar view as in Fig. 3, but showing the injury to the hepatic vein to the LL lobe (arrow). The LL lobe has been retracted down and and the right of the image to show the injury site.

I. OVERVIEW
Date: July 10, 2014
Swine no: 227
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 07/08/2014
Pre-procedure wt: 33.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 70
- MAP: 130
- Temp: 36.8
- EtCO2: 30

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:40 AM
Spleen wt: 276 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 276 + 20 + 123.6 = 419.6 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 50 = 950 mL

Pre-injury VS
- HR: 72
- MAP: 110
- Temp: 34.4
- EtCO2 : 19

III. INJURY & TREATMENT PHASE

Time of injury: 09:04 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 90
Resuscitation fluid: warm LR, 3.38 L preset maximum (100 mL/kg), given at constant rate of 18.8 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 09:05AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:14 AM

10 min post-injury VS
- HR: 98
- MAP: 29
- Temp: 34.4
- EtCO2: 14

Blood draw no. 3: (30 min post-injury): 09:34 AM

30 min VS
- HR: 1108
- MAP:
- Temp: 34.2
- EtCO2: 18

Blood draw no. 4: (60 min post-injury): 10:04 AM

60 min VS
- HR: 96
- MAP: 87
- Temp: 33.9
- EtCO2: 22

Blood draw no. 5: (90 min post-injury): 10:34 AM

90 min VS
- HR: 94
- MAP: 62
- Temp: 33.8
- EtCO2: 21

Blood draw no. 6: (120 min post-injury): 11:04 AM

120 min VS
- HR: 91
- MAP: 68
- Temp: 33.7
- EtCO2: 20

Blood draw no. 7: (150 min post-injury): 11:34 AM

150 min VS
- HR:89
- MAP: 74
- Temp: 33.7
- EtCO2: 20

Blood draw no. 8: (180 min post-injury): 11:38 AM

180 min VS
- HR: 87
- MAP: 62
- Temp: 33.8
- EtCO2: 19

Survival at 180 min? Yes
Target MAP attained ? Yes, very briefly.
Time of death: 12:04 PM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 428.4 mL (suction) + 793.5 mL (clot + lap pads) = 1221.9 mL
- IV fluid given: LR (37°C): 4050 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft (see Figs). Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe.

Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 858.8 g

Tissue harvested: none

VI. COMMENTS

Standard noncompress injury, no treatment, restricted IVF resuscitation. Easy survival to 180 min, with only 1.2 L blood loss.

Subject was spontaneously hypoventilating prior to injury (breathing on his own over the ventilator), forcing his EtCO2 down to 20 on his own. We seemed to offset this a little by increasing the isoflurane and adjusting increasing the tidal volume and vent rate. There was a cuff leak on the ET tube, perhaps this was the cause. But we did get him to settle down a little prior to the injury. Not sure if this had any impact on the post-injury course.

Tally so far with the no treatment/restricted IVF is 5 survivals, 1 death.

VII. PLAN

Swine 228, next in series, on Friday July 11, 2014.

Figure 1, swine 227. Lateral view of abdomen just prior to injury. Heart rate = 71, O2 sat = 86%, endtidal pCO2 = 20, MAP = 111, temp = 34.4. Cephalad is to the right.


Figure 2, swine 227. Lateral view of abdomen at 175 min after injury. Subject alive, with heart rate = 88, O2 sat = 98%, endtidal pCO2 = 18, MAP = 60, temp = 33.8. Cephalad is to the right.

Figure 3, swine 227. Overhead view of re-opened abdomen 180 min after injury; subject subject alive, MAP ~40. Moderate amount free blood & clot, mostly in upper abdominal cavity. Cephalad is to the right.
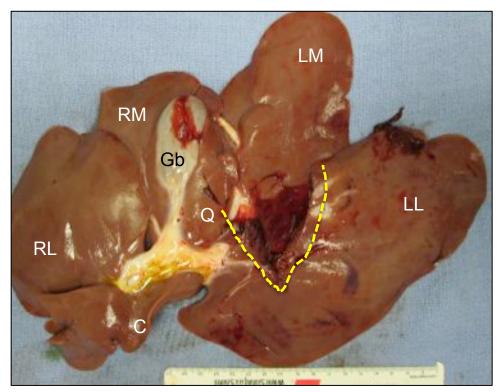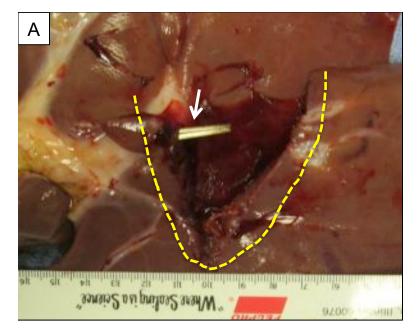

Figure 4, swine 227. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Gb = gallbladder. Large clot covering injury site (arrow).
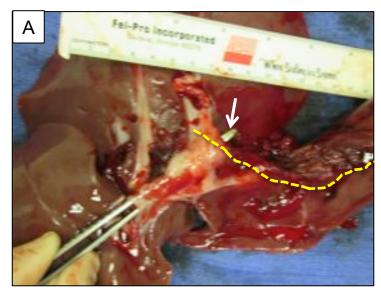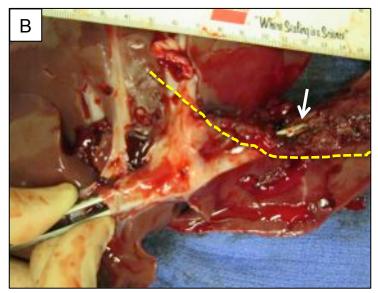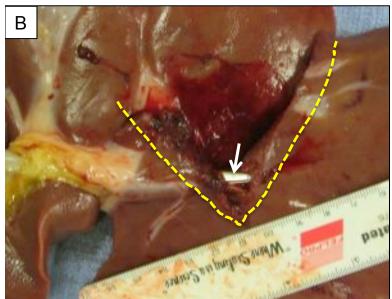
Figure 5, swine 227. Liver *ex vivo,* inferior aspect, showing injury site. Subject had transection of 2 portal vein branches and 1 hepatic vein branch.
(A) Anterior portal vein branch transection. Forceps emerging from cut end of PV branch (arrow).
(B) (B) Posterior portal vein branch transection. Forceps emerging from cut end of PV branch (arrow).
(C) Transected HV, showing distal end on the left lateral lobe (arrow) .

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I. OVERVIEW
Date: July 11, 2014
Swine no: 228
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: female (gilt)
Date swine received from UNL Mead: 07/08/2014
Pre-procedure wt: 33.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 117
- MAP: 103
- Temp: 37.2
- EtCO2: 36

Blood draw no. 1 (initial): 8:40 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:32 AM
Spleen wt: 320 gm
LR (22°C) infused after splenectomy: mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 320 + 20 + 25.5 = 365.5 mL
- LR (22°C) infused (spleen replacement + incidental): 960 + 50 = 1010 mL

Pre-injury VS
- HR: 111
- MAP: 102
- Temp: 35.5
- EtCO2 : 45

III. INJURY & TREATMENT PHASE

Time of injury: 08:52 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.

Treatment formulation: No treatment.

Clotting factors: None

Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 3.4 L preset maximum (100 mL/kg), given at constant rate of 19 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:53AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:02 AM

10 min post-injury VS
- HR: 128
- MAP: 38
- Temp: 35.2
- EtCO2: 24

Blood draw no. 3: (30 min post-injury): 09:22 AM

30 min VS
- HR: 151
- MAP: 27
- Temp: 34.8
- EtCO2: 20

Survival at 180 min? No
Target MAP attained ? No
Time of death: 09:45 AM
Cause of death: exsanguination from injury
Interval from injury to death: 53 min

Post-treatment fluid data:
- Blood loss: 737.8 mL (suction) + 548.4 mL (clot + lap pads) = 1286.2 mL
- IV fluid given: LR (37°C): 1050 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate
    amounts of blood and some clots are seen (see Figs). No clot was covering the injury site (see Figs).
Heart: no clots/no air present upon opening the right ventricle.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1.5 branches, to LL lobe. Second portal vein branch injury was a partial tangential injury.
Other: none
Ex vivo total liver wt: 794.1 g

Tissue harvested: none

VI. COMMENTS

Subject expired at 53 min. Only lost 1.2 L blood. Interestingly, last subject which was very close in
weight also lost 1.2 L blood, but survived easily to 180 min.
Today's subject was female. We had intended all subjects be male for this study; today's female sneaked
through the delivery process from the Mead ARDC, unintentionally. I don't think the sex of the subject should
matter that much, but since today subject (a female) died rather easily, I'm not so sure.
Tally is now 5 survivals, 2 deaths.

VII. PLAN

At least three more subjects using the same procedure. Next one will be on Friday, July 18th, 2014. s
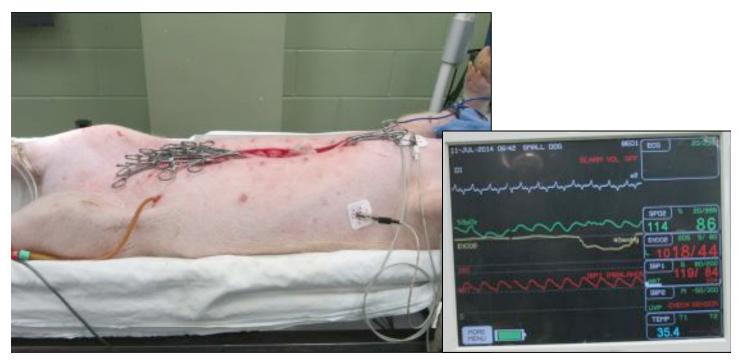
Figure 1, swine 228. Lateral view of abdomen just prior to injury. Heart rate = 114, O2 sat = 86%, endtidal pCO2 = 44, MAP = 104, temp = 35.4. Cephalad is to the right.
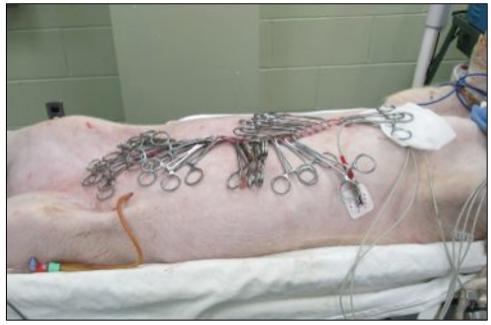
Figure 2, swine 228. Lateral view of abdomen at 53 min after injury, just after expiration (MAP ~7, EtCO2 = 0). Cephalad is to the right.

Figure 3, swine 228. Overhead view of re-opened abdomen after expiration. Moderate amount free blood & clot, in upper & lower abdominal cavity. Cephalad is to the right.

Figure 4, swine 228. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder. Injury site without significant clot.

Figure 5, swine 228. Liver *ex vivo,* inferior aspect, showing injury site. Subject had full transection of 1 portal vein branch, partial transection of another branch, and full transection of 1 hepatic vein branch.

(A) Anterior portal vein branch transection, full transection. Forceps emerging from cut end of PV branch (arrow).

(B) (B) Posterior portal vein branch transection, partial transection. Forceps emerging from tangential injury of PV branch (arrow).

(C) Transected HV, showing distal end on the left lateral lobe (arrow); oblique lateral view.

Figures, Swine 228, p. 3 of 3

I. OVERVIEW
Date: July 24, 2014
Swine no: 231
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: male
Date swine received from UNL Mead: 07/16/2014
Pre-procedure wt: 37.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
  1. Endotracheal tube with ETCO2 monitor
  2. EKG clips
  3. Left ear vein angiocath (20g) for supplemental LR
  4. Right carotid artery angiocath (20g), cutdown; for BP monitor
  5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
  6. Transabdominal cystotomy for 16 Fr Foley catheter
  7. Rectal temp probe
  8. Pulse oximetry
  9. Heating pad below subject

Initial VS
  • HR: 81
  • MAP: 97
  • Temp: 37.1
  • EtCO2: 14

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:45 AM
Spleen wt: 181.5 gm
LR (22°C) infused after splenectomy: 800 mL at 150 mL/min

Pre-injury fluid data:
  • Blood loss (spleen weight + phlebotomies + incidental): 181.5 + 20 + 152 = 353.5 mL
  • LR (22°C) infused (spleen replacement + incidental): 800 + 50 = 850 mL

Pre-injury VS
  • HR: 80
  • MAP: 82
  • Temp: 36
  • EtCO2 : 24

III. INJURY & TREATMENT PHASE

Time of injury: 08:58 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.

Treatment formulation: No treatment.

Clotting factors: None

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 65

Resuscitation fluid: warm LR, 3.38 L preset maximum (100 mL/kg), given at constant rate of 20.6 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:59AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:09 AM

10 min post-injury VS
- HR: 159
- MAP: 15
- Temp: 35.3
- EtCO2: 7

Blood draw no. 3: (30 min post-injury): 09:29 AM

30 min VS
- HR: 162
- MAP: 12
- Temp: 35.3
- EtCO2: 3

Survival at 180 min? No
Target MAP attained ? No.
Time of death: 09:32 AM
Cause of death: exsanguination from injury
Interval from injury to death: 34 min

Post-treatment fluid data:
- Blood loss: 1272.2 mL (suction) + 753.1 mL (clot + lap pads) = 2025.3 mL
- IV fluid given: LR (37°C): 1100 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen. There was no clot covering injury site (see Figs).
Heart: Clear blood and no clots or air seen in right ventricle upon opening.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe. A tangential injury along its long axis, approx 1 cm in length.
Other: none
Ex vivo total liver wt: 796.4 g

Tissue harvested: none

VI. COMMENTS

Rapid death after a standard injury to the LL lobe. Subject was relatively hypotensive from the start (pre-injury MAP ~80) compared to our typical pig. No cardiac embolism. Death does appear to secondary to exsanguination. Tally with the restricted IVF resuscitation is now 5 survivals, 3 deaths.

VII. PLAN

Repeat procedure in next swine, no. 232, on July 25th.
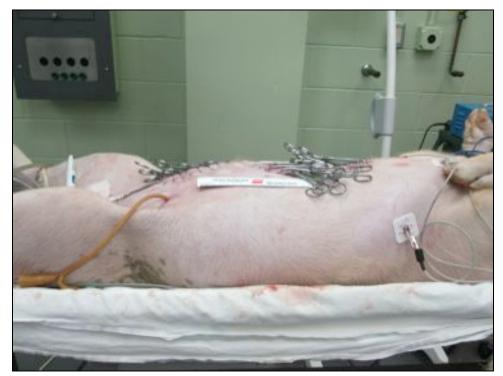
Figure 1, swine 231. Lateral view of abdomen at 34 min after injury, just after expiration (MAP ~5, EtCO2 = 0). Cephalad is to the right.
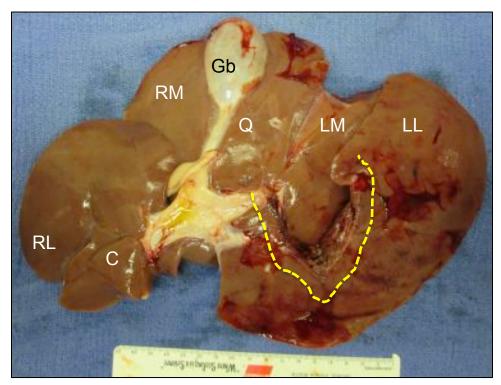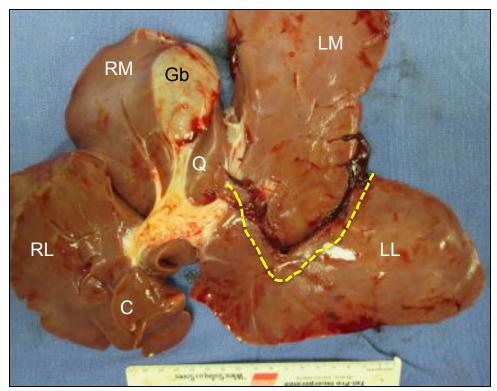

Figure 2, swine 231. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder. Injury site did not have any clot.
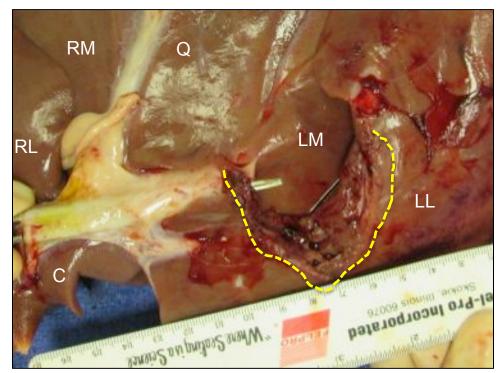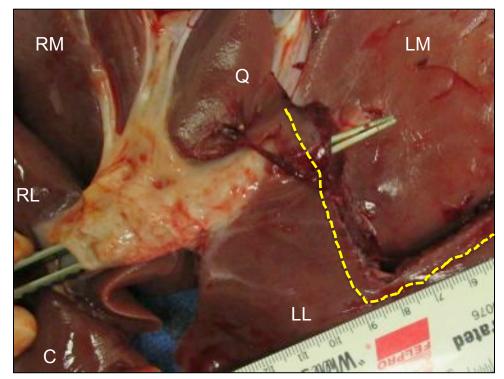
Figure 3, swine 231. Liver *ex vivo,* inferior aspect, showing injury site, close-up of Fig. 2. Forceps shown emerging from transected branch of portal vein to LL lobe.
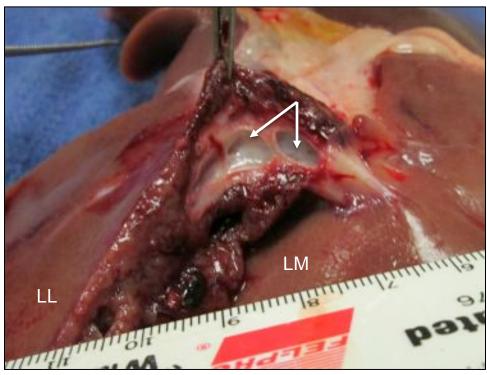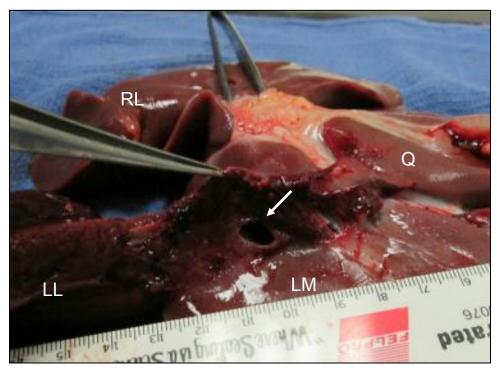

Figure 4, swine 231. Liver *ex vivo,* inferior lateral aspect, showing injury site. The white arrows indicate the transected branch of the portal vein to the LL lobe. This branch appears to have been cut along its axis, resulting in a long, widely patent opening.
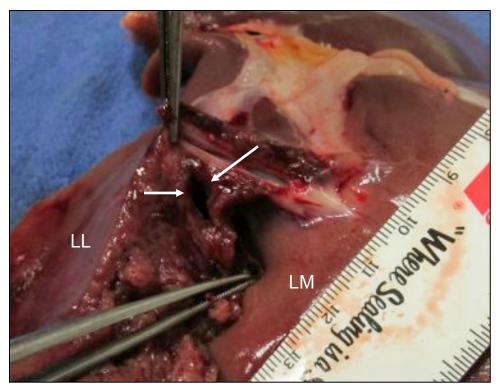
Figure 5, swine 231. Liver *ex vivo,* inferior lateral aspect, showing injury site, similar view as in Fig. 4. The white arrows indicate the transected branch of the relatively small hepatic vein to the LL lobe.

I. OVERVIEW
Date: July 25, 2014
Swine no: 232
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:25 AM
Swine sex: male
Date swine received from UNL Mead: 07/16/2014
Pre-procedure wt: 38.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
 1. Endotracheal tube with ETCO2 monitor
 2. EKG clips
 3. Left ear vein angiocath (20g) for supplemental LR
 4. Right carotid artery angiocath (20g), cutdown; for BP monitor
 5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
 6. Transabdominal cystotomy for 16 Fr Foley catheter
 7. Rectal temp probe
 8. Pulse oximetry
 9. Heating pad below subject

Initial VS
 • HR: 93
 • MAP: 95
 • Temp: 37.6
 • EtCO2: 45

Blood draw no. 1 (initial): 7:45 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:04 AM
Spleen wt: 458.9 gm
LR (22°C) infused after splenectomy: 1400 mL at 150 mL/min

Pre-injury fluid data:
 • Blood loss (spleen weight + phlebotomies + incidental): 458.9 + 20 + 0 = 478.9 mL
 • LR (22°C) infused (spleen replacement + incidental): 1400 + 50 = 1450 mL

Pre-injury VS
 • HR: 102
 • MAP: 111
 • Temp: 36.3
 • EtCO2 : 45

- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:15 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 90
Resuscitation fluid: warm LR, 3.38 L preset maximum (100 mL/kg), given at constant rate of 21.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:16AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:25 AM

10 min post-injury VS
- HR: 141
- MAP: 16
- Temp: 35.8
- EtCO2: 12

Blood draw no. 3: (30 min post-injury): 08:45 AM

30 min VS
- HR: 1108
- MAP:
- Temp: 34.2
- EtCO2: 18

Survival at 180 min? No
Target MAP attained ? No.
Time of death: 08:45 AM

Cause of death: exsanguination from injury
Interval from injury to death: 30 min

Post-treatment fluid data:
- Blood loss: 1407.5 mL (suction) + 656.9 mL (clot + lap pads) = 2064.4 mL
- IV fluid given: LR (37°C): 600 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen. A moderate sized clot was seen near the injury site, but not occluding the transected vessels (see Figs).
Heart: Clear blood without any clots or air was seen coming out of right ventricle upon opening.
Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 1 branch, to LL lobe
Other: none
Ex vivo total liver wt: 831.3 g

Tissue harvested: none

VI. COMMENTS

Rapid death (within half hour) after a standard injury to the LL lobe. Subject was normotensive pre-injury compared to our typical pig. No cardiac embolism. Death does appear to secondary to exsanguination. Tally with the restricted IVF resuscitation is now 5 survivals, 4 deaths.

When we have reached N = 10 with this protocol (i.e., one more subject), we will tally all the data and compare the outcomes to the group of 10 pigs that received rapid LR resuscitation. At this point the differences between the groups do not seem as large as I had anticipated, but we'll see how the data pans out.

VII. PLAN

The tenth subject will be done on Friday, Aug 1st 2014 at 8 AM.

Figure 1, swine 232. Lateral view of abdomen at 30 min after injury, just after expiration (MAP ~5, EtCO2 = 0). Cephalad is to the right.

Figure 2, swine 232. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder. Injury site had a small amount of adherent clot.
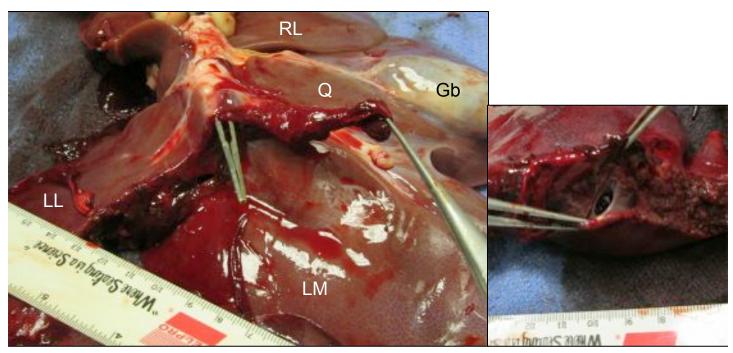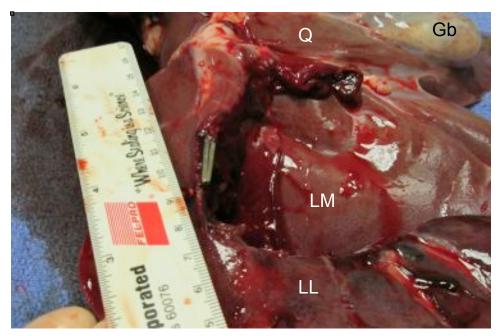
Figure 3, swine 231. Liver *ex vivo,* inferior aspect, showing injury site, close-up of Fig. 2. Forceps shown emerging from transected branch of portal vein to LL lobe.

Figure 4, swine 232. Liver *ex vivo,* inferior lateral aspect, showing injury site. The white arrow indicates the transected branch of the hepatic vein to the LL lobe.

I. OVERVIEW
Date: August 1, 2014
Swine no: 235
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: male
Date swine received from UNL Mead: 07/30/2014
Pre-procedure wt: 36.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 87
- MAP: 106
- Temp: 37.1
- EtCO2: 36

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:20 AM
Spleen wt: 413.3 gm
LR (22°C) infused after splenectomy: 1240 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 413.3 + 20 + 0 = 433.3 mL
- LR (22°C) infused (spleen replacement + incidental): 1240 + 50 = 1290 mL

Pre-injury VS
- HR: 108
- MAP: 101
- Temp: 35.6
- EtCO2 : 38

III. INJURY & TREATMENT PHASE

Time of injury: 08:36 AM

Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes, but directed onto the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.

Treatment formulation: No treatment.

Clotting factors: None

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 75

Resuscitation fluid: warm LR, 3.64 L preset maximum (100 mL/kg), given at constant rate of 20.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.

Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:37AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:46 AM

10 min post-injury VS
- HR: 93
- MAP: 52
- Temp: 35.2
- EtCO2: 31

Blood draw no. 3: (30 min post-injury): 09:06 AM

30 min VS
- HR: 85
- MAP: 57
- Temp: 34.9
- EtCO2: 30

Blood draw no. 4: (60 min post-injury): 09:36 AM

60 min VS
- HR: 87
- MAP: 54
- Temp: 34.4

- EtCO2: 30

Blood draw no. 5: (90 min post-injury): 10:06 AM

90 min VS
- HR: 84
- MAP: 52
- Temp: 34.2
- EtCO2: 27

Blood draw no. 6: (120 min post-injury): 10:36 AM

120 min VS
- HR: 80
- MAP: 56
- Temp: 33.4
- EtCO2: 28

Blood draw no. 7: (150 min post-injury): 11:06 AM

150 min VS
- HR: 95
- MAP: 57
- Temp: 33.9
- EtCO2: 15

Blood draw no. 8: (180 min post-injury): 11:36 AM

180 min VS
- HR: 114
- MAP: 51
- Temp: 33.7
- EtCO2: 32

Survival at 180 min? Yes
Target MAP attained ? No.
Time of death: 11:36 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 606.2 mL (suction) + 788.6 mL (clot + lap pads) = 1394.8 mL
- IV fluid given: LR (37°C): 4600 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering injury site (see Figs).
Heart: not examined.

Number of hepatic veins lacerated: 1, to LL lobe.
Portal vein injury: 2 branches, to LL lobe
Other: none
Ex vivo total liver wt: 1062.7 g

Tissue harvested: none

VI. COMMENTS

Subject no. 235 had a standard injury and survived quite easily to 3 h. We have now completed N = 10 subjects who underwent the standard noncompressible injury with no intraabdominal treatment, resuscitated with the slow IVF rate ("hypotensive resuscitation"). The survival breakdown after the 180 min observation period was 6 survivors and 4 deaths. This 60% survival is exactly what the survival was with the previous control group, which I was not expecting, but I suspect there will be a number of differences once we have analyzed all the numerical data.

VII. PLAN

Analyze numerical data, compare to previous control group, formulate a plan to move forward with the noncompressible hemorrhage project. Next procedure date pending this analysis.
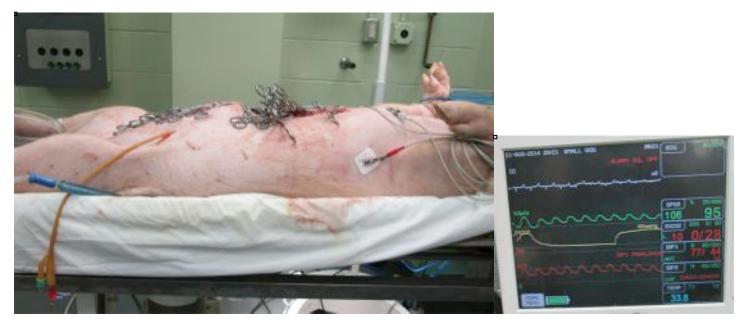
Figure 1, swine 235. Lateral view of abdomen at 180 min after injury. Subject is alive and well with HR = 106, MAP ~58, O2 sat = 95%, EtCO2 = 28, and T = 33.8°C.
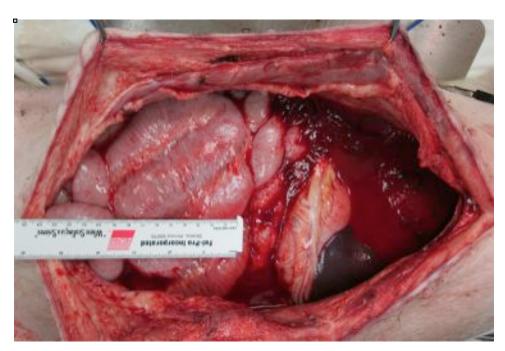
Figure 2, swine 235. Overhead view of abdomen reopened immediately after 180 min observation period. Subject still alive with MAP ~40. Cephalad is to the right. Moderate amount clot and unclotted blood in abdomen.
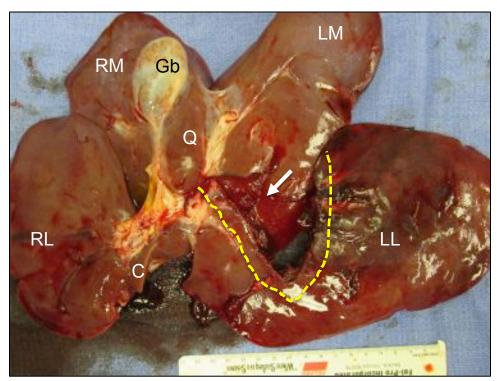
Figure 3, swine 235. Liver *ex vivo,* inferior aspect, showing injury site. Dashed yellow line = gap in LL lobe induced by cut. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; C = caudate lobe; Q = quadrate lobe; Gb = gallbladder. Injury site had adherent clot (arrow).

Figure 4, swine 235. Liver *ex vivo,* inferiolateral aspect, showing injury site, close-up of Fig. 3. Forceps on left shown emerging from transected branch of portal vein to LL lobe; forceps on right pulling away clot that was adherent to injury site. Inset: close-up of transected portal vein branch on distal side of LL lobe injury (area corresponds to that underneath the ruler in the larger image).

Figure 5, swine 235. Liver *ex vivo,* inferiolateral aspect, showing injury site, another close-up of Fig. 3. Forceps shown emerging from a second transected branch of portal vein to LL lobe (subject had two PV branches transected).

Figure 6, swine 235. Liver *ex vivo,* inferior lateral aspect, showing close-up of injury site. The white arrow indicates the transected branch of the hepatic vein to the LL lobe*.*

I. OVERVIEW
Date: September 17, 2014
Swine no: 237
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 08:10 AM
Swine sex: female
Date swine received from UNL Mead: 07/30/2014
Pre-procedure wt: 55.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 57
- MAP: 118
- Temp: 37.8
- EtCO2: 29

Blood draw no. 1 (initial): 8:50 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:10 AM
Spleen wt: 498.0 gm
LR (22°C) infused after splenectomy: 1494 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 498 + 20 + 111.8 = 629.8 mL
- LR (22°C) infused (spleen replacement + incidental): 1494 + 50 = 1544 mL

Pre-injury VS
- HR: 81
- MAP: 122
- Temp: 35.7
- EtCO2 : 34

III. INJURY & TREATMENT PHASE

Time of injury: 09:35 AM
Injury type: hepatic left medial "lobectomy," nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR, 2.8 L preset maximum (50 mL/kg), given at constant rate of 15.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 09:36AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:45 AM

10 min post-injury VS
• HR: 112
• MAP: 18
• Temp: 35.3
• EtCO2: 12

Blood draw no. 3: (30 min post-injury): 10:05 AM

30 min VS
• HR: 92
• MAP: 15
• Temp: 35.2
• EtCO2: 12

Blood draw no. 4: (Final; 50 min post-injury): 10:25 AM

Survival at 180 min? No
Target MAP attained ? No.
Time of death: 10:25 AM
Cause of death: exsanguination from injury

Interval from injury to death: 50 min

Post-treatment fluid data:
- Blood loss: 1376.1 mL (suction) + 655.2 mL (clot + lap pads) = 2031.3 mL
- IV fluid given: LR (37°C): 810 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering the surface of the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 182.2 (resected LM lobe) + 945.7 (remaining liver) = 1127.9g

Tissue harvested: IVC

VI. COMMENTS

First subject with new noncompressible injury mechanism (left medial liver lobectomy). This subject was larger than typically utilized, because this subject was a unused from another protocol.

This injury mechanism provides a large surface area that faces anteriorly (Figure 4), such that it should be in direct contact with injected foam. This is in contrast to the previous noncompressible injury mechanism (hemitransection of left lateral liver lobe near its base), which produced a bleeding site that was not in direct contact with the injected foam. Per our recent discussions, we thought that an injury that was more "anterior" in location might be more useful for the foam testing than the posteriorly-located injury that we were using.

Subject died from apparent exsanguination at 50 min. The subject developed profound hypotension very early (MAP = 18 at 10 min), but technically survived (measurable arterial pulse wave and $EtCO_2$) well past this point. The hemorrhage from this injury obviously is quite severe, in that large-diameter portal and hepatic veins (Figure 7) bleed completely unimpeded into the free peritoneal space. Bleeding from the previous mechanism was somewhat impeded because of its posterior location.

If we find that complete transection of the left medial lobe at its base is too severe an injury, then we can always perform the transection several cm distal/peripheral from the present location (i.e., further out on the liver lobe), presumably where the major venous branches will have a smaller diameter.

VII. PLAN

Repeat protocol in Swine 238.

Figure 1, swine 237. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).

Figure 2, swine 237. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy on the inferior side of the LM lobe is indicated by the position of the ruler. View from the hindlimbs up to the head (inferior view).
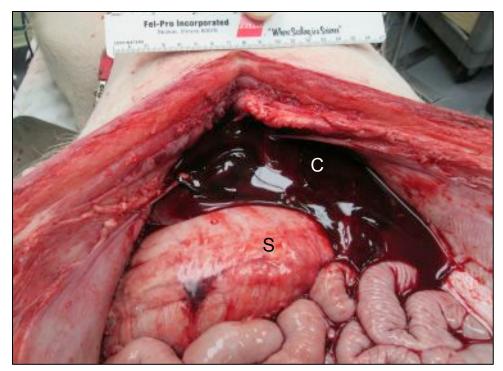
Figure 3, swine 237. Site of injury, after death of subject. A large clot (C) overlies the injury site (latter not visible). Cephalad is at top of image. S = stomach.
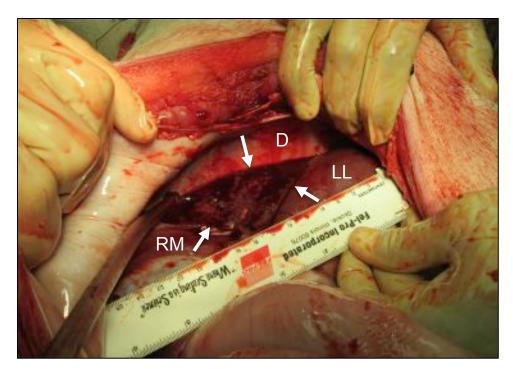
Figure 4, swine 237. Site of injury, after death of subject. The clot in Figure 3 has been removed, exposing the surface of the injury site (arrows). Cephalad is at top of image. D = diaphragm; LL = left lateral lobe of liver; RM = right medial lobe of liver.
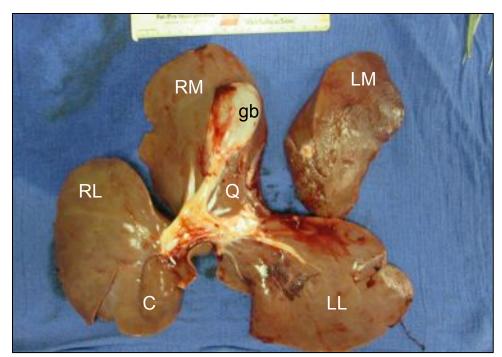
Figure 5, swine 237. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Anterior is at top of image.
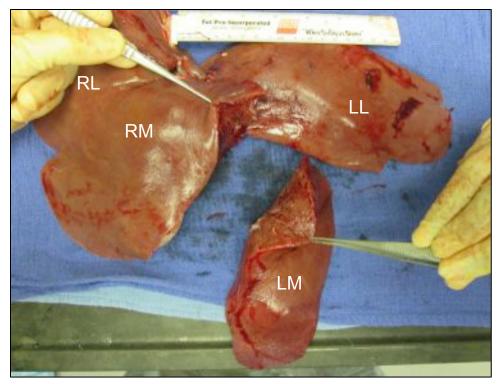
Figure 6, swine 237. Liver *ex vivo*, superior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral. Anterior is at bottom of image.

Figures, Swine 237, p. 3 of 4
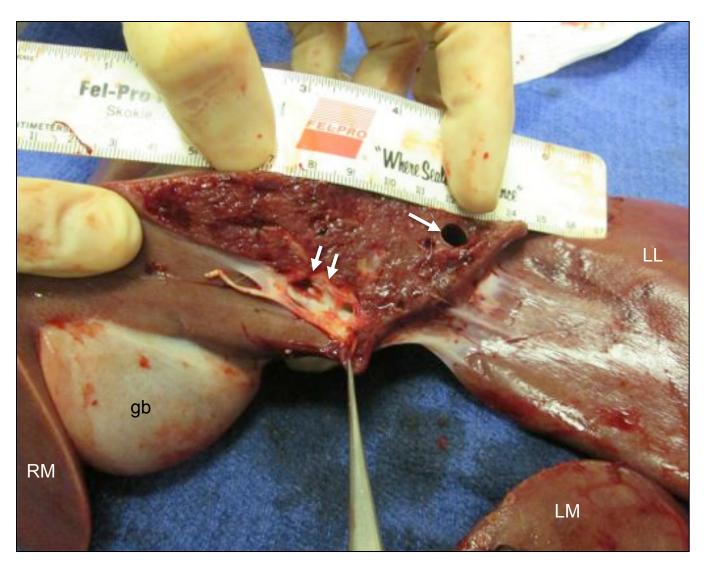
Figure 7, swine 237. Liver *ex vivo*, close-up of injury/lobectomy site. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates transected hepatic vein to LM lobe; double arrow indicated transected portal vein branch to LM lobe.

I. OVERVIEW
Date: September 19, 2014
Swine no: 238
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 09/15/2014
Pre-procedure wt: 34.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 93
- MAP: 79
- Temp: 36.1
- EtCO2: 42

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 237.5.0 gm
LR (22°C) infused after splenectomy: 712.5 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 237.5 + 20 + 30 = 287.5 mL
- LR (22°C) infused (spleen replacement + incidental): 712.5 + 50 = 762.5 mL

Pre-injury VS
- HR: 93
- MAP: 131
- Temp: 35.8
- EtCO2 : 42

III. INJURY & TREATMENT PHASE

Time of injury: 08:50 AM
Injury type: hepatic left medial "lobectomy," nonanatomical (see Figures). The left medial lobe of the liver was
   transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the
   lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial)
   was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as
   described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the
   upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 105
Resuscitation fluid: warm LR, 1.7 L preset maximum (50 mL/kg), given at constant rate of 9.5 mL/min,
   continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive
   resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at
   T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:51AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:00 AM

10 min post-injury VS
   • HR: 116
   • MAP: 55
   • Temp: 35.6
   • EtCO2: 36

Blood draw no. 3: (30 min post-injury): 09:20 AM

30 min VS
   • HR: 110
   • MAP: 48
   • Temp: 35.4
   • EtCO2: 32

Blood draw no. 4: (Final; 60 min post-injury): 10:25 AM

60 min VS
   • HR: 105
   • MAP: 11
   • Temp: 34.2
   • EtCO2: 7

Survival at 180 min? No
Target MAP attained ? No
Time of death: 09:55 AM
Cause of death: exsanguination from injury
Interval from injury to death: 65 min

Post-treatment fluid data:
- Blood loss: 1057.2 mL (suction) + 641.6 mL (clot + lap pads) = 1698.8 mL
- IV fluid given: LR (37°C): 550 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering the surface of the liver at the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 195.2 (resected LM lobe) + 771.1 (remaining liver) = 966.3g

Tissue harvested: IVC

VI. COMMENTS
        Subject (N = 2) was no-treatment control with revised noncompressible injury mechanism (complete transection of left medial liver lobe at its base). This subject survived to 1+ h; blood loss was 1.7 L. The early profound hypotension seen in the previous subject was not seen in this subject. In fact, the course followed in the exsanguination of this subject seemed to be fairly ideal with respect to what we would like to seen in a model such as this; i.e., severe, ultimately fatal hemorrhagic injury, but not so severe that the animal expires with 15-20 min despite any intervention.
        Note: the post-injury IVF restriction has been capped at 50 mL/kg.

VII. PLAN

        We will need an N = 6-10 of no-treatment controls such as this.
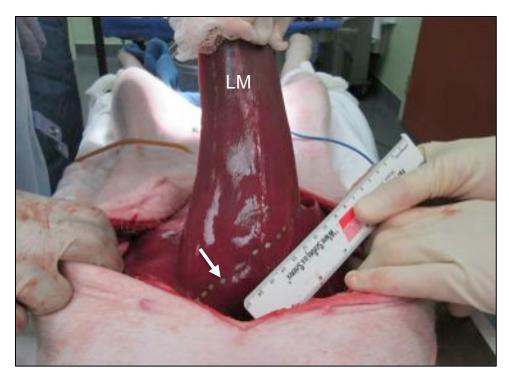
Figure 1, swine 238. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).
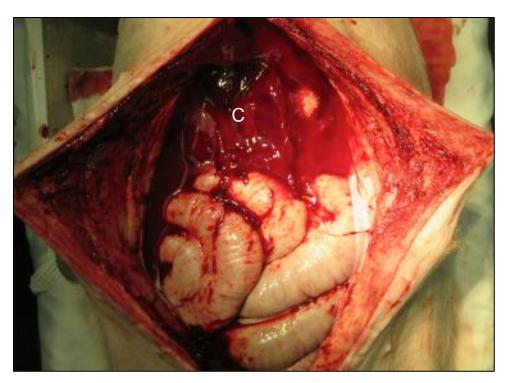
Figure 2, swine 238. Site of injury, after death of subject. A large clot (C) overlies the injury site (latter not visible). Loops of intestine are visible inferior to the clot. Cephalad is at top of image.
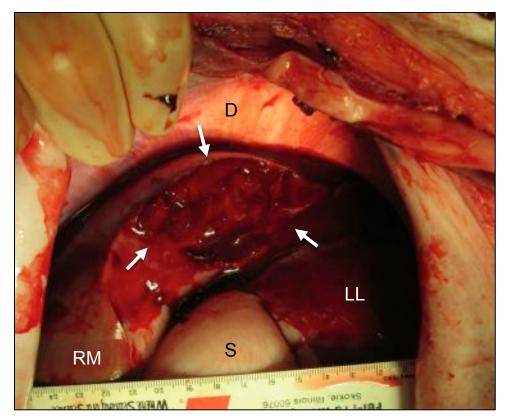
Figure 3, swine 237. Site of injury *in situ*, after death of subject. The clot in Figure 2 has been removed, exposing the surface of the injury site (arrows). Cephalad is at top of image. D = diaphragm; LL = left lateral lobe of liver; RM = right medial lobe of liver; S = stomach.
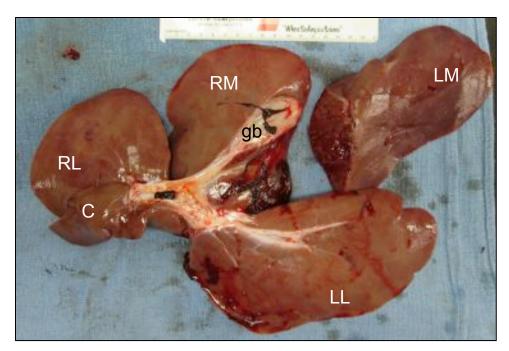
Figure 4, swine 238. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate. gb = gallbladder. Anterior is at top of image.

Figure 5, swine 238. Liver *ex vivo*, superior surface, close-up of Figure 4. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral, reflected medially. A layer of clot (C) is covering the injury site. Anterior is at bottom of image.
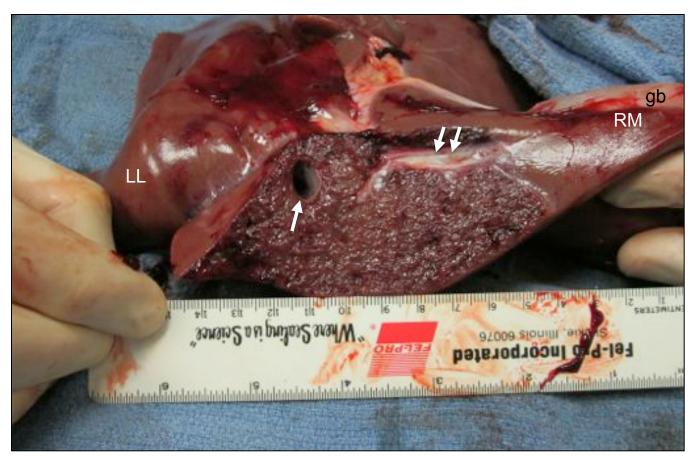
Figure 6, swine 238. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). Clot in Figure 5 has been removed. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates transected hepatic vein to LM lobe; double arrow indicated transected portal vein branch to LM lobe.

I. OVERVIEW
Date: September 23, 2014
Swine no: 239
Model: swine, normothermic, normovolemic, liver lobe resection
Treatment: suture & cautery control of bleeding
Personnel: Carlson, Yanala, Hansen, Siford

II. PRE-RESECTION PHASE

Start time: 08:00 AM
Swine sex: female
Date swine received from UNL Mead: 09/15/2014
Pre-procedure wt: 30.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 85
- MAP: 116
- Temp: 38.3
- EtCO2: 39

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 281.8.0 gm
LR (22°C) infused after splenectomy: 845 mL at 150 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 281.8 + 20 + 0 = 301.8 mL
- LR (22°C) infused (spleen replacement + incidental): 845 + 25 = 870 mL

Pre-resection VS
- HR: 78
- MAP: 116
- Temp: 36.5
- EtCO2 : 34

III. RESECTION & TREATMENT PHASE

Time resection began: 09:10 AM
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was resected using a combination of cautery and suture control of prominent vessels. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Bandage treatment: none.
Clotting factors: none
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 95
Resuscitation fluid: warm LR given at constant rate of 15.0 mL/min (wt in kg/2 = mL/min), continuously during the entire 60 min observation period, or until animal expires. Begun at T = 0 min (T = time resection completed and specimen removed from abdomen).
Time resection completed and resuscitation fluid begun: 09:15 AM

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:25 AM

15 min post-injury VS
- HR: 72
- MAP: 89
- Temp: 35.9
- EtCO2: 29

Blood draw no. 3: (Final; 60 min post-injury): 10:10 AM

60 min VS
- HR: 72
- MAP: 87
- Temp: 35.2
- EtCO2: 27

Survival at 60 min? YES
Target MAP attained ? No
Time of death: 10:10 AM
Cause of death: intentional exsanguination from euthanasia (transect supradiaphragmatic IVC)
Interval from injury to death: 60 min

Post-treatment fluid data:
- Blood loss: 100 mL (suction) + 92.5 mL (clot + lap pads) = 192.5 mL
- IV fluid given: LR (37°C): 775 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, small amounts of blood and some clots are seen (see Figs). A small clot was covering the surface of the injury site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 123.0 (resected LM lobe) + 705.5 (remaining liver) = 828.5g

Tissue harvested: IVC

VI. COMMENTS

First subject tested in the resumption of the NEDED project using a large (>100 g) liver resection. This was a "control" resection, in that the bleeding was handled with clamping/tying, suture ligatures, and electrocautery. Blood loss from the resection portion of the procedure (including the 60 min observation period) was 190 mL, which I think is fairly reasonable. This might decrease as we perform more of these control resections. I am planning on an N of 10 for this control group. We will do five now, then test some bandages, and then do five more controls at a later date.

Note: I am using a "reasonable" fluid rate for post-resection resuscitation, empirically determined by dividing the wt (in kg) by half, and then using this number as the mL/min for the LR. So a 30 kg subject would receive 900 mL of LR in 1 h.

VII. PLAN

Next control resection scheduled for Friday Sep 26, 2014.
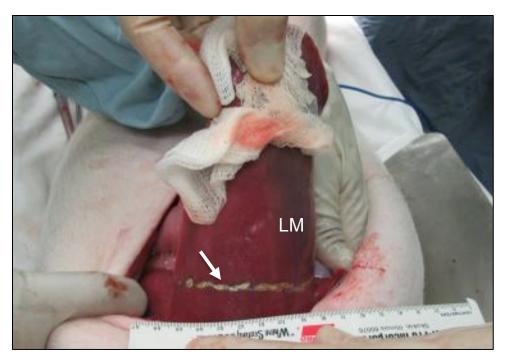
Figure 1, swine 239. Site of planned resection on left medial (LM) liver lobe. The LM lobe has been exteriorized out of the midline incision. The planned plane of resection has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).
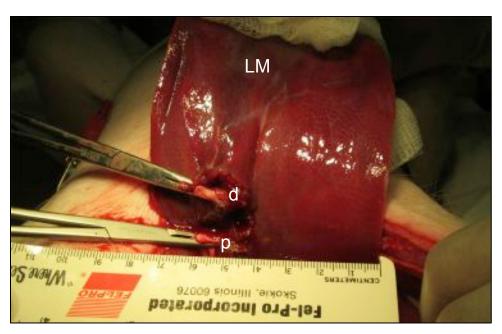
Figure 2, swine 239. Process of resection. The main portal vein branch to the LM lobe has been isolated and clamped proximally (p) & distally (d). View from the hindlimbs up to the head (inferior view).
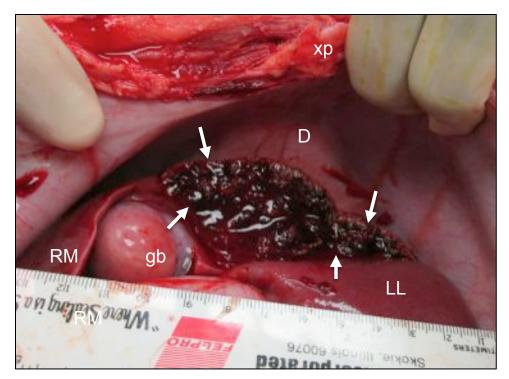
Figure 3, swine 239. Site of resection *in situ*, 60 min after specimen removed; subject alive and well with MAP = 85. Arrows indicate transected surface of LM lobe. Cephalad is at top of image. D = diaphragm; LL = left lateral lobe of liver; RM = right medial lobe of liver; gb = gallbladder; xp = xiphoid process.
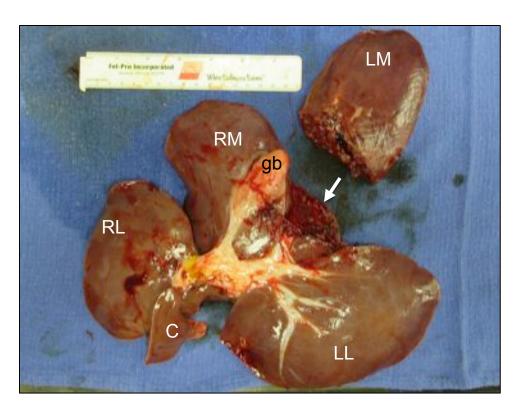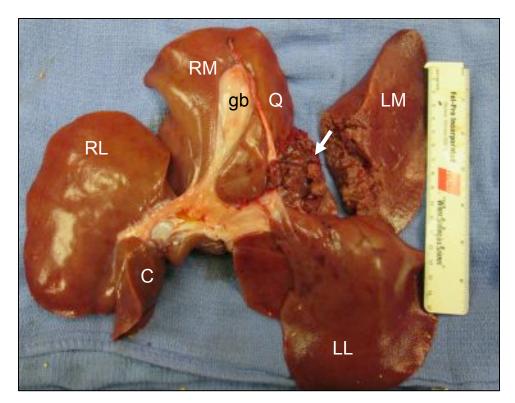

Figure 4, swine 239. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate. gb = gallbladder. Arrow = transected surface of LM lobe. Anterior is at top of image.
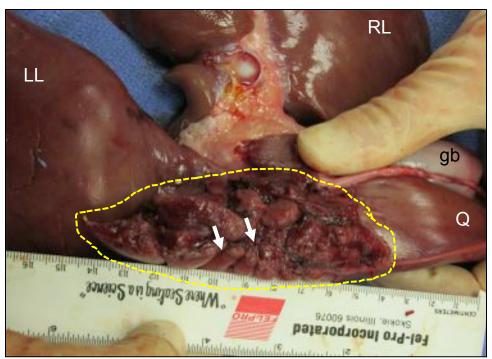
Figures, Swine 239, p. 2 of 3
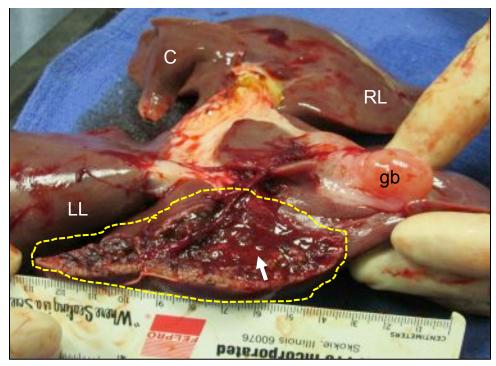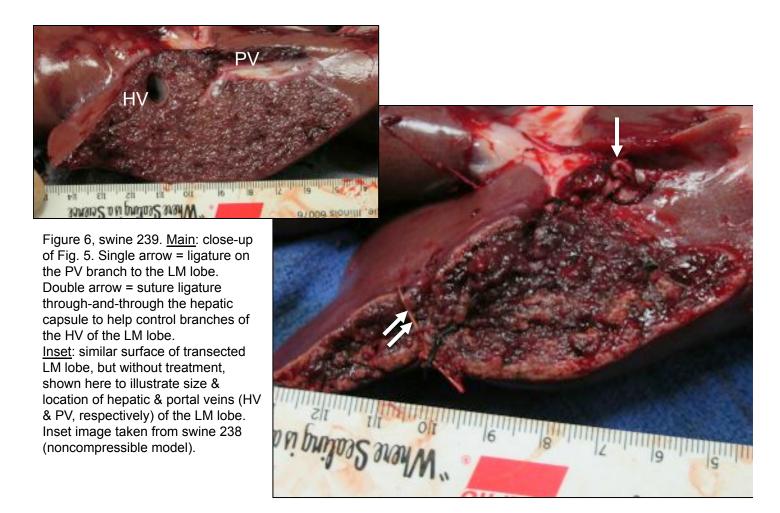
Figure 5, swine 239. Liver *ex vivo*, left inferior aspect. Liver lobes indicated: RL = right lateral; LL = left lateral, reflected medially. gb = gallbladder. Resection site outline with yellow dashed line. A layer of clot (arrow) partially covers the resection site. Anterior is at bottom of image.

Figure 6, swine 239. Main: close-up of Fig. 5. Single arrow = ligature on the PV branch to the LM lobe. Double arrow = suture ligature through-and-through the hepatic capsule to help control branches of the HV of the LM lobe.
Inset: similar surface of transected LM lobe, but without treatment, shown here to illustrate size & location of hepatic & portal veins (HV & PV, respectively) of the LM lobe. Inset image taken from swine 238 (noncompressible model).

I. OVERVIEW
Date: September 26, 2014
Swine no: 240
Model: swine, normothermic, normovolemic, liver lobe resection
Treatment: suture & cautery control of bleeding
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-RESECTION PHASE

Start time: 08:05 AM
Swine sex: male
Date swine received from UNL Mead: 09/15/2014
Pre-procedure wt: 34.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 136
- MAP: 88
- Temp: 38.5
- EtCO2: 56

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:38 AM
Spleen wt: 213.3 gm
LR (22°C) infused after splenectomy: 640 mL at 150 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 213.3 + 20 + 0 = 233.3 mL
- LR (22°C) infused (spleen replacement + incidental): 640 + 25 = 665 mL

Pre-resection VS
- HR: 93
- MAP: 96
- Temp: 37.4
- EtCO2 : 44

III. RESECTION & TREATMENT PHASE

Time resection began: 08:50 AM
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was resected using a combination of parenchymal finger fracture, cautery, suture control of prominent vessels, and completion oversew of the resection site using a #1 Vicryl on a liver needle. Prior to the resection, the lower half of the ventral midline incision was closed with towel clips. The resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Bandage treatment: none.
Clotting factors: none
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 75
Resuscitation fluid: warm LR given at constant rate of 17.0 mL/min (wt in kg/2 = mL/min), continuously during the entire 60 min observation period, or until animal expires. Begun at T = 0 min (T = time resection completed and specimen removed from abdomen).
Time resection completed and resuscitation fluid begun (t = 0): 09:00 AM
Blood loss during resection: (suction + clots + sponges): 369.3 + 0 + 182.6 = 551.9 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 09:15 AM

15 min post-resection VS
- HR: 81
- MAP: 51
- Temp: 36.8
- EtCO2: 40
- IAP: 0

Blood draw no. 3: (Final; 60 min post-resection): 10:00 AM

Final (60 min) VS
- HR: 74
- MAP: 56
- Temp:36.1
- EtCO2: 38

Survival at 60 min? Yes
Target MAP attained ? No.
Time of death: 10:00 AM
Cause of death: intentional exsanguination from euthanasia (transect supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 49.1 mL (suction) + 0 mL (clot + lap pads) = 49.1 mL
- IV fluid given: LR (37°C): 1200 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, small
    amounts of blood and some clots are seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 128.4 (resected LM lobe) + 832.9 (remaining liver) = 961.3g

Tissue harvested: IVC for practice harvest of ECs

VI. COMMENTS

2nd subject tested in the NEDED project using a large (>100 g) liver resection ("control", in which the
bleeding was handled with clamping/tying, suture ligatures, and electrocautery). Blood loss from the resection
portion of the procedure (including the 60 min observation period) was ~600 mL, which was a bit more than the
1st subject. A reasonable blood loss for this procedure probably will be in the 300-400 mL range. Still planning on
an N of 10 for this control group. We will do five now, then test some bandages, and then do five more controls at
a later date.

VII. PLAN

Continue next week with more controls. Dates tba.

Figure 1, swine 240. Site of resection *in situ*, 60 min after specimen removed; subject alive with MAP = 55. Arrows indicate transected surface of LM lobe. Cephalad is at top of image. D = diaphragm; LL = left lateral lobe of liver; RM = right medial lobe of liver; xp = xiphoid process.

Figure 2, swine 240. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral (reflected posteriorly); C = caudate; Q = quadrate. gb = gallbladder. Arrow = transected surface of LM lobe. Anterior is at top of image.

Figure 3, swine 240. Liver *ex vivo*, left inferior aspect. Liver lobes indicated: RL = right lateral; LL = left lateral, reflected medially. gb = gallbladder. Resection site outline with yellow dashed line. Anterior is at bottom of image. Compression sutures of #1 Vicryl are visible (arrows).

A totally recombinant human fibrin sealant

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ARTICLE INFO

ABSTRACT

Background: Applications of plasma-derived human fibrin sealants (pdhFS) have been limited because of cost, limited supply of pathogen-screened plasma, the need for bioengineering improvements, and regulatory issues associated with federal approval. We describe a totally recombinant human fibrin sealant (rhFS), which may engender an abundant, safe, and cost-effective supply of efficacious fibrin sealant.

Materials and methods: A first-generation rhFS made from recombinant human fibrinogen (rhFI; produced in the milk of transgenic cows), activated recombinant human factor XIII (rhFXIIIa; produced in yeast), and recombinant human thrombin (rhFIIa; purchased, made in animal cell culture) was formulated using thromboelastography (TEG). The hemostatic efficacy of rhFS *versus* commercial pdhFS was compared in a nonlethal porcine hepatic wedge excision model.

Results: The maximal clot strength of rhFS measured *in vitro* by TEG was not statistically different than that of pdhFS. TEG analysis also showed that the rhFS gained strength more quickly as reflected by a steeper α angle; however, the rhFS achieved this clot strength with a 5-fold lower factor I content than the pdhFS. When these fibrin sealants were studied in a porcine hepatic wedge excision model, the hemostatic scores of the rhFS were equivalent or better than that of the pdhFS.

Conclusions: The bioengineered rhFS had equivalent or better hemostatic efficacy than the pdhFS in a nonlethal hemorrhage model, despite the factor I concentration in the rhFS being about one-fifth that in the pdhFS. Because the rhFS is amenable to large-scale production, the rhFS has the potential to be more economical and abundant than the pdhFS, while having a decreased risk of blood-borne pathogen transmission.

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1. Introduction

The use of dried plasma as a topical hemostatic aid was documented in 1909 [1]. The combination of relatively pure fibrinogen (factor I or FI) with thrombin to make fibrin glue or foam was described in 1944 [2], but it was not until improved purification technology became available that fibrin sealants (FS) became commercially available in the 1970s [1]. Since that time, the efficacy of FS products as a topical hemostat or tissue adhesive has been demonstrated in numerous elective clinical

scenarios, including peripheral vascular procedures [3], total knee arthroplasty [4], reoperative cardiac procedures [5], pulmonary resection [6], bleeding duodenal ulcer [7], and partial nephrectomy [8]. FS alone was not useful during hepatectomy [9], but FS combined with a collagen matrix applied during liver resection reduced blood loss and/or postoperative drainage compared with standard operative care [10]. Examples of currently available FS formulations that use plasma-derived fibrinogen include Evicel (Ethicon, Inc; Somerville, NJ) and Tisseel (Baxter Healthcare; Deerfield, IL).

The United States Department of Defense has maintained an interest in the development of hemostatic devices using FS for control of traumatic hemorrhage [11]. There has been particular interest in hemostatic FS devices for use under coagulopathic conditions [12]. Topical hemostatic treatments that incorporate FS have been successfully used in porcine trauma models, including femoral vessel injury [13], aortic injury [14], and hepatic injury [15]. One notable FS-containing device for traumatic hemorrhage was the Dry Fibrin Sealant Dressing, produced by the American Red Cross [16]. The Dry Fibrin Sealant Dressing was efficacious in porcine models of lethal hemorrhage [12,17] and was anecdotally successful in military trauma but was discontinued due to fragility and cost issues [18].

The availability of a relatively abundant FS might increase innovation into FI-based hemostatic devices for the treatment of severe hemorrhage. The essential components of FS are: FI, the biomonomer from which fibrin polymer is made [19]; activated thrombin (factor IIa or FIIa), which catalyzes the formation of soluble fibrin from FI and also activates factor XIIIa [20]; and activated factor XIII (FXIIIa), which cross-links the fibrin polymer to itself (rendering it insoluble) and to the wound surface [21]. One abundant source for these clotting factors can be large-scale recombinant protein production.

The complexity of FI and FIIa necessitates that recombinant versions of these proteins be made in animal cells [22]. We recently reported the production of recombinant human FI (rhFI) made at high concentrations in the milk of dairy cows [23]. Recombinant human FIIa (rhFIIa) already is commercially available (Recothrom; ZymoGenetics, Inc, Seattle, WA) and has been approved for topical hemostatic therapy in the United States and in Europe [24]. In contrast to FI and FIIa, FXIIIa is less complex; its core catalytic unit (FXIIIA2), which is kinetically faster than the more complex tetrameric plasma-borne FXIII [21], has been produced at large scale in yeast. FXIII nomenclature and specific activity are summarized in Table 1. Recombinant FXIIIA2 (rFXIIIA2) currently is in clinical studies of FXIII replacement therapy [25]. In the present study, we used a porcine hepatic wedge resection model to compare the hemostatic efficacy of a fully recombinant human FS (rhFS), containing rhFI, rhFIIa, and recombinant human FXIIIa (rhFXIIIa), against a commercially available, plasma-derived human FS (pdhFS).

2.   Materials and methods

2.1.   Animal studies

The use of swine was approved by the Subcommittee of Animal Studies and by the Research and Development Committee at the Omaha VA Medical Center. The number of swine ($n = 6$)

Table 1 – Sources of factor XIII activity.

Factor XIII species	Abbreviation	Activity (U/mg)
Plasma-derived tetrameric factor XIII	FXIII	40[*]; 6–8[†]
Plasma-derived, dimeric, catalytic subunit factor XIII	FXIIIA2	N/A
Recombinant dimeric catalytic subunit factor XIII	rFXIIIA2	140[‡]
Plasma-derived, activated, dimeric factor XIII	FXIIIA2a	N/A
Recombinant human, activated factor XIII	rhFXIIIa	7000[§]

N/A = not applicable.
[*] Activity based on normal plasma pool, which by definition is 1 U/mL.
[†] Reported activity of plasma FXIII [37].
[‡] Reported activity of FXIIIA2 made in *Saccharomyces cerevisiae* [25].
[§] Reported activity of rFXIIIa made in *Pichia pastoris* [23].

used for each group in the two-group comparison of rhFS *versus* pdhFS (with hemostatic score as the outcome measurement) was determined with a statistical power analysis [26], using Δ/σ (Cohen d, in which Δ is the desired difference in means set by the observer and σ is the estimated standard deviation) = 2.0, false-positive rate (α) = 0.05, false-negative rate (β) = 0.2, and power (π, or $1 - \beta$) = 0.8.

2.2.   Clotting factor sources

rhFI was produced in the milk of transgenic cows by inserting the primary sequence of the human transgenes for the α-, β-, and γ-chains of fibrinogen into the cow genome by nuclear transfer [23]. Southern blot analysis confirmed the presence of the three transgenes. The rhFI expressed in the milk of the transgenic cows was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, γ and γ'-chain content, fibrinopeptide phosphorylation, glycosylation, thrombin-catalyzed activation, thrombin-catalyzed protofibril formation, factor XIIIa–catalyzed molecular cross-linking, viscoelasticity, scanning electron microscopy, and tissue sealant function [23]. The main differences between rhFI made in transgenic cow milk and plasma-derived fibrinogen (pdFI) was the γ'-chain content [23].

The human FXIIIA1 gene was expressed in *Pichia pastoris* [23,27]. The expressed rhFXIIIa was characterized by SDS-PAGE, Western blot, Pefakit FXIII incorporation assay, FXIIIa-catalyzed molecular cross-linking of fibrin, and viscoelasticity [27]. Human rhFIIa (Recothrom) was purchased from ZymoGenetics, Inc. Human pdFI depleted of plasminogen, von Willebrand factor, and fibronectin was purchased from Enzyme Research Laboratories (South Bend, IN). Commercial human pdhFS (Tisseel, unless otherwise specified) was purchased from Baxter BioSurgery (Deerfield, IL).

2.3.   Determination of clotting factor concentration and activity

The concentrations of the purified stocks of rhFI, pdFI, and rFXIIIa were determined by OD_{280} and the bicinchoninic acid
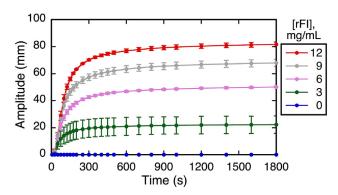
Fig. 1 – Thromboelastographic dose-response analysis of rhFI. Samples with constant concentrations of pdFIIa (53 U/mL) and rFXIIIa (2400 U/mL) were tested with varying concentrations of rhFI (0—12 mg/mL). Shown are the results from a single experiment with triplicate tubes; data are expressed as mean ± standard deviation. (Color version of figure is available online.)

assay [28]. The specific activity of rFXIIIa was estimated to be 7000 U/mg using the Pefakit FXIII Incorporation Assay (Pentapharm, Norwalk, CT).

2.4. Thromboelastography

The effect of rhFI concentration on clot formation kinetics and strength (Fig. 1) was determined with thromboelastography (TEG), using a TEG 5000 Thrombelastograph (Haemonetics Corp, Braintree, MA). rhFI (0—12 mg/mL) was incubated at 37°C with rhFIIa (53 U/mL), rFXIIIa (2400 U/mL), and CaCl$_2$ (12 mM). For the comparison of rhFS versus pdhFS, the latter was prepared as per the manufacturer's instructions by mixing the sealant and thrombin solutions in equal proportions in the TEG assay cup at 37°C. For the rhFS, rhFI (9 mg/mL) was incubated at 37°C in the cup with rhFIIa (106 U/mL), rFXIIIa (2500 U/mL), and CaCl$_2$ (12 mM). The thromboelastograph was calibrated each day of use; each time point of each analysis was run in triplicate. TEG Analytical Software (version 4.2.2; Haemonetics Corporation, Braintree, MA) was used to calculate the time to clot initiation (R), time to clot firmness of 20 mm (K), alpha angle (α), maximal clot strength (maximal amplitude [MA], which was directly related to the shear elastic modulus strength, G), and percent lysis 60 min after MA (LY60) [29]. A single-factor analysis of variance was performed to compare the effect of plasmin on pdhFS and rhFS. The Student t-test (two-tailed, with unequal variances) was used to compare rhFS and pdhFS, with alpha set at 0.05.

2.5. Immunoblotting

Immunoblotting was used to estimate the mass concentrations of FXIII in the commercial pdhFS (Tisseel). Reduced samples were resolved with SDS-PAGE on 4%—12% NuPage Bis-Tris gels (Invitrogen, Carlsbad, CA) and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA). Blots were probed with polyclonal antibodies for FXIII

(F0019-46; United States Biological, Swampscott, MA) or FIIa (T5045-10B; United States Biological).

2.6. Swine hepatic injury models

Domestic swine (castrated males, aged 3 mo, weight 33—36 kg) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska—Lincoln. Each subject was fasted for 12 h before surgery, but with free access to water. Each subject was premedicated with Telazol (4.4 mg/kg; Zoetis, Madison, NJ), ketamine (2.2 mg/kg), and xylazine (2.2 mg/kg) as a single intramuscular injection. An intravenous line was established in an auricular vein, oral endotracheal intubation was performed, and anesthesia was maintained with 0.5%—1.5% isoflurane using a Matrx VMS veterinary anesthesia machine (Midmark Corp, Versailles, OH). Mechanical ventilation was maintained at 12—15 breaths/min with a tidal volume of 10—15 mL/kg, to keep the end-tidal pCO$_2$ at 30—35 mm Hg. A heating pad was under each subject to support body temperature. A carotid arterial catheter was placed for pressure monitoring and blood sampling, and a jugular venous catheter was placed for isotonic fluid and medication administration, all via a surgical cutdown in the left neck. Arterial pressure, end-tidal pCO$_2$, rectal temperature, cardiac electrical activity, and pulse oximetry were continuously recorded with a Bionet BM5 Veterinary Monitor (Bionet America, Inc, Tustin, CA) interfaced to a laptop computer. Each swine subject was maintained under an appropriate level of isoflurane anesthesia (indicated by the absence of corneal reflex) for the duration of the experiment; before euthanasia, the isoflurane was increased (see the following sections).

The porcine normothermic nondilutional stellate liver laceration model was adapted from a previous description [17]. After the above initial setup, a ventral midline incision was made, splenectomy was performed, and a transabdominal cystostomy tube was placed. Splenectomy generally has been performed in severe porcine hemorrhage models to eliminate the confounding effects of splenic autotransfusion [30]. A liver laceration was created with a custom-built liver injury clamp (Fig. 2A, inset), which consisted of tines in an X-configuration (5 cm diameter) on one arm of the clamp, and a base plate on the other arm onto which the tines seat. The base plate was placed on the inferior surface of the liver against the quadrate lobe, between the cystic duct and the portal vein. The tines were positioned over the liver dome, 4—5 cm anterior to the vena cava at the base of the left medial hepatic segment. The clamp then was closed, forcing the tines through the liver dome and onto the base plate. The test sealant (10 mL) then was applied immediately into the laceration, and the edges of the laceration were held together with manual compression for 5 min. Only one clamp application per subject was performed. No other hemostatic maneuver (e.g., cotton gauze, laparotomy packs, Pringle maneuver) was used other than sealant with manual compression. This model was intended for preliminary qualitative observation of sealant efficacy only. Quantitative data (blood loss volume, mean arterial pressure, and so forth) was not recorded for the stellate laceration model.

For the wedge excision model, a ventral midline incision was made and a cystostomy tube was placed. The left lobe of
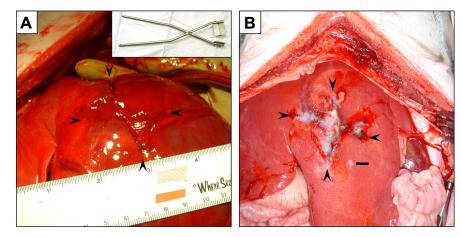
Fig. 2 – Treatment of porcine hepatic stellate laceration with human FS. The liver is shown *in situ* exposed through a ventral midline incision, immediately after FS application to the injury; top of each image is cephalad. (A) pdhFS treatment; the liver injury clamp is shown in the inset. (B) rhFS treatment (Table 2 formulation). Bar = 1 cm; arrowheads indicate peripheral extent of the stellate hole made by the clamp tines. (Color version of figure is available online.)

the liver was exteriorized, and sites for wedge-shaped ("pie-slice") hepatic excisions were lightly scored with electrocautery on the liver capsule (Fig. 3A) along the anterior edge of both the left medial and left lateral segment (two separate but identical series of wedge excisions per subject). The base of each excision (i.e., distance along the lobar edge) was maintained constant at 1 cm. The excision depth (i.e., distance from lobar edge to apex of excision; Fig. 3A) of each series ranged from 0.5 to 3.0 cm, in crescendo fashion using 0.5 cm increments, for a total of six excisions per series, all cut with scissors (Supplementary Video File). Two such series of excisions were performed per subject, for a total of 12 excisions per subject. Only one type of sealant (either the rhFS or pdhFS) was used in any given subject.

Each excision was treated immediately with up to 1 mL of FS without digital or gauze compression or other adjunct (Supplementary Video File). pdhFS (Tisseel) was administered with the double-barrel common-channel syringe system (Duploject, Baxter Healthcare; Fig. 3C) provided by the manufacturer, as per the user instructions. The rhFS (as defined in Table 2) was administered using two 1 mL syringes taped together, with 30 gauge needles bent into convergence (Fig. 3D). One syringe contained the rhFI and rFXIIIa, and the other syringe contained the rhFIIa and calcium.

Hemostasis 30 s after treatment of each excision was scored as follows: 0 = failure/minimal hemostasis; 1 = decreased but steady bleeding; 2 = oozing; and 3 = hemostasis. Quantification of the small amount of blood loss, which occurred during the treatment phase, did not produce reliable data, so the above visual analog score of hemostasis was used. If an excision was not hemostatic 30 s after application of 1 mL of the test sealant, then the excision was packed with cotton gauze before performing the next excision, to minimize the ongoing blood loss. Excisions were performed during a single nonsurvival anesthetic, which lasted for 30–45 min. After completion of the two series of excisions, each subject was administered 5% isoflurane for 3 min, the supradiaphragmatic inferior vena cava then was transected, and each subject was allowed to expire from exsanguination while under deep isoflurane anesthesia.

2.7. Fibrinogen immunohistochemistry

Liver specimens were fixed in 10% neutral-buffered formalin, dehydrated, and then embedded in paraffin blocks. Paraffin sections (5 μm) underwent antigen retrieval with Target Retrieval Solution (Dako North America, Carpinteria, CA) as per the manufacturer's instructions. The presence of both human and swine fibrinogen were detected by sequential dual-immunohistochemical staining followed by a hematoxylin counterstain. Signal detection was performed using the Dako EnVision G/2 Doublestain System (Dako North America, Inc, Carpinteria, CA), as per the manufacturer's instructions. Briefly, endogenous peroxidase and alkaline phosphatase activity present in the tissue were blocked and then the swine fibrinogen antibody (Kamiya Biomedical Company, Seattle, WA) was applied and visualized using the horseradish peroxidase/3,3′-diaminobenzidine tetrahydrochloride reagents from the Dako system (Dako North America, Inc). A second blocking step was performed before incubation with the human fibrinogen antibody (Abcam, Inc, Cambridge, MA). The human fibrinogen antibody was visualized using alkaline phosphatase–based Permanent Red (Dako North America), as per the manufacturer's instructions.

3. Results

3.1. Effect of FI on clot formation

The effect of rhFI concentration (0–12 mg/mL) on the kinetics and strength of clot formation with rhFS was analyzed by TEG (Fig. 1). The speed of clot formation, as measured by clot initiation time (R), time to clot firmness (K), and angle (α), increased as the rhFI concentration was increased to 6 mg/mL but remained constant >6 mg/mL of rhFI (R = 17 s, K = 50 s, α = 82°). The maximal clot strength (MA) continued to increase with each increase of rhFI concentration (up to 80 mm with rhFI = 12 mg/mL). For further *in vivo* studies, we targeted an rhFI concentration of 9 mg/mL in the rhFS, which generated
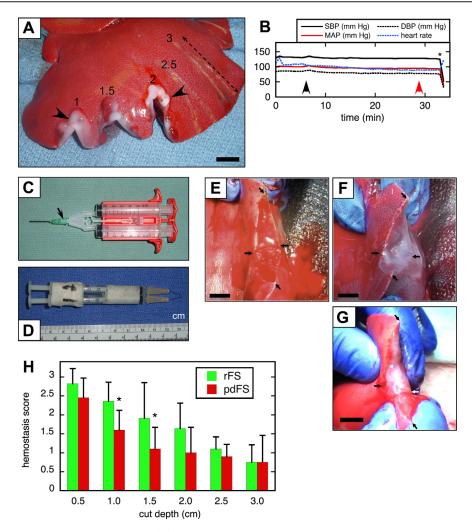
Fig. 3 — Porcine hepatic wedge excision model. (A) Intraoperative view of anterolateral edge of left lateral liver lobe during an excision procedure; top of picture is cephalad. The numbers above each excision indicate cut depth in centimeters. Excisions 1, 1.5, and 2 already were cut and treated with rhFS (arrowheads indicate white clot of sealant); excisions 2.5 and 3 were scored with cautery but had not yet been cut. Excision "depth" is dimension indicated by dashed line. (B) Vital sign recording during a typical procedure. DBP = diastolic blood pressure; MAP = mean arterial pressure; SBP = systolic blood pressure; black arrowhead = start of procedure; red arrowhead = completion of procedure; *euthanasia (inferior vena cava transection). (C) Proprietary dual-chamber syringe device (Duploject) for administration of the commercial pdhFS. Arrow indicates common mixing channel. (D) Improvised double syringe assembly for administration of the rhFS. (E) Individual wedge cut of 2 cm depth, immediately before treatment. Arrows indicate corners of excision (excision depth may appear >2 cm because some distraction occurs during handling). (F) Wedge cut in (B) immediately after treatment with rhFS. (G) A separate 1-cm deep wedge cut, immediately after treatment with commercial pdhFS. Bars = 1 cm. (H) Hemostasis scores of hepatic wedge excisions treated with rhFS (Table 2 formulation) versus commercial pdhFS. Each bar represents mean score ± standard deviation of 10—12 excisions; taller bar = better hemostasis. *P < 0.05, Wilcoxon signed-rank test. (Color version of figure is available online.)

MA >50 mm. At constant rhFI concentration, the calcium concentration (3—15 mM) did not affect the thromboelastographic parameters of the rhFS (data not shown).

3.2.   Thromboelastography of pdhFS versus rhFS

The formulation of the first-generation rhFS formulation is compared with commercial pdhFS (Tisseel) in Table 2. The concentration of fibrinogen was about 5-fold greater in the pdhFS with respect to the rhFS. Furthermore, the pdhFS contained ~2-fold higher level of FIIa than the rhFS. The rhFS, however, had ~50-fold higher FXIII activity compared with

the pdhFS. When evaluated by TEG (Table 3), the rhFS had significantly faster clotting kinetics compared with pdhFS, as measured by the angle of clot development (α) (Table 3); the maximal clot strength (MA) was not statistically different ($P = 0.059$) nor were the R and K values.

3.3.   Treatment of stellate liver laceration with rhFS versus pdhFS

Preliminary experiments with the sealants in a porcine liver laceration model suggested that the rhFS had better hemostatic efficacy compared with the commercial pdhFS

Table 2 — Comparison of component concentration and activity in pdhFS versus rhFS.

Component	pdhFS	rhFS
Fibrinogen (FI)	34—53 mg/mL	9 mg/mL
Thrombin (FII)	200—313 U/mL[*]	106 U/mL
Factor XIII/XIIIa		
Concentration	<0.1 mg/mL[†]	0.36 mg/mL
Activity	10—50 U/mL[‡]	2475 U/mL
$CaCl_2$	18—22 μM	12 mM
Fibrinolysis inhibitor	2250—3750 KIU/mL	0

KIU = Kallikrein Inhibitor Unit.
[*] Tisseel package insert information.
[†] Based on immunoblot analysis (data not shown).
[‡] As reported on www.rxmed.com [38].

(Fig. 2). Injection of 10 mL of pdhFS into a stellate laceration of the liver dome followed by 5 min of manual compression resulted in persistent hemorrhage (Fig. 2A); on the other hand, hemostasis was achieved with the rhFS treatment (Fig. 2B; N = 2 swine per sealant). Subjectively, the white clot generated by the rhFS set up quicker, appeared more opaque, and felt more adherent than the clot generated by the pdhFS. Postmortem examination of the liver *ex vivo* demonstrated that one large hepatic vein was injured in each subject; there were no major portal, biliary, or hepatic artery injuries.

3.4. rhFS versus pdhFS in the hepatic wedge excision model

To determine whether there were differences in hemostatic efficacy between rhFS and pdhFS, the hemostasis assay in Figure 3, involving small hepatic wedge excisions, was devised expressly for this study. The primary determinant in this assay was the hemostatic action of the sealant alone; that is, there would be no external compression of the bleeding surfaces by the surgeon. In brief, wedges cut were made along the edge of a liver lobe (Fig. 3A), treated with a defined amount of sealant, and then a visual analog hemostasis score was assigned. Preliminary work with this model demonstrated that bleeding from an excision with a 0.5-cm cut depth was easy to control with sealant alone, whereas bleeding from 3-cm excision was quite difficult to control. So a range of

Table 3 — TEG parameters of pdhFS (commercial) and rhFS.

Fibrin sealant	R (s)	K (s)	α (°)	MA (mm)
rhFS	10.33 ± 0.75	50 ± 0.0	85.07 ± 1.12[*]	63.15 ± 12.12
pdhFS	9.44 ± 1.36	54.45 ± 8.67	81.66 ± 2.93	77.41 ± 5.49

α = kinetics of clot development; K = achievement of 20 mm clot strength (amplitude); MA = maximal amplitude (maximum clot strength); R = reaction time (first evidence of clot formation).
Values are mean ± standard deviation of five and six separate experiments on multiple batches of rhFS (formulation in Table 2) and commercial pdhFS (Tisseel), respectively.
[*] $P = 0.034$ compared with pdhFS, unpaired t-test.

excisions with a stepwise increase in cut depth (0.5—3 cm) was chosen as a discriminator of hemostatic efficacy. This range (or series) of excisions was performed twice in each subject, on separate liver lobes during the same anesthetic. Each subject tolerated these procedures well with <150 mL blood loss and minimal to no perturbation in vital signs (Fig. 3B). TEG of blood drawn immediately before *versus* after procedure completion did not demonstrate any deterioration of clotting during the excisions (done in all subjects; data not shown).

The intended technique of FS delivery was to use a double-chamber single-channel syringe (a proprietary device for delivery of the pdhFS; Fig. 3C) in all subjects. This device keeps the fibrinogen and the activated thrombin in separate chambers; when the operator depresses the linked syringe plungers, the protein solutions are mixed in a common channel and then ejected from the syringe tip. Use of this device with the proprietary pdhFS produced a reasonably consistent stream of sealant with occasional clogging of the tip. Initial attempts at delivery of the rhFS with this proprietary double-chamber single-channel syringe, however, resulted in tip clogging before adequate rhFS could be delivered to the wound. To deliver the rhFS to the wound, the improvised delivery system shown in Figure 3D was used. Two tuberculin-type syringes were taped together, and the needles were bent to produce a convergent stream. This syringe setup moved the mixing of the rhFS components from inside the syringe assembly to outside on the wound surface, which enabled rhFS delivery at a volume and flow subjectively equivalent to that obtained with the pdhFS.

The subjective impression from treating hepatic wedge excisions with rhFS *versus* pdhFS confirmed the earlier observation from the hepatic laceration model, that is, the clot produced by the rhFS set up quicker, was more opaque, and was more tenacious/adherent than the clot produced by the pdhFS (Fig. 3A,F,G). A comparative analysis of hemostatic efficacy of these two sealants in the hepatic wedge excision model is shown in Figure 3H. Overall, the hemostatic scores of the rhFS were equivalent or better than those of the pdhFS. At cut depths of 1.0 and 1.5 cm, the rhFS had greater hemostatic efficacy than the pdhFS; there was a nonsignificant trend of greater efficacy at the 2.0 cm depth. At cut depth ≥2.5 cm, the hemorrhage mostly was too brisk to control with either sealant in the absence of any extrinsic compression (Fig. 3H).

3.5. Immunohistochemistry

Double immunohistochemistry of porcine and human FI in treated wedge excisions is shown in Figure 4. The fibrin cap over the hepatic wound surface typically was thicker in the rhFS sections compared with the pdhFS sections. These double-stained sections also demonstrated the mixing of the native porcine fibrin (brown color) with human (plasma-derived or recombinant) fibrin (red color) over the surface of the wound. In both the pdhFS- and rhFS-treated samples, the predominant FI in the clot appeared to be endogenous in origin (i.e., porcine). Additional images of hepatic wedge excisions treated with rhFS have been supplied in Supplementary Figure S1.

Fig. 4 — Factor FI immunohistochemistry of porcine hepatic wedge excision ± human FS treatment. Liver sections were double-stained for porcine (brown) and human (red) fibrinogen, with hematoxylin counterstain. (A and D) No treatment. (B and E) pdhFS treatment. (C and F) rhFS treatment. Bars: A—C = 1 mm, D—F = 200 μm; c = clot; hp = hepatic parenchyma. (Color version of figure is available online.)

4.   Discussion

Our intention in the design of this first-generation rhFS was to achieve clotting speed and strength comparable with an available pdhFS formulation, as determined by TEG. The commercial pdhFS used in this report had a clot onset of <10 s and maximal clot strength of 50—70 mm displacement. To obtain a clot onset of <10 s, 106 U/mL of rhFIIa was used (somewhat less than the level of FIIa contained in the pdhFS; Table 2). To obtain a maximal clot strength of >50 mm, 2500 U/mL of rFXIIIa were used. We believe that by providing a relatively large amount of rFXIII in its activated form, we produced higher rates of cross-linked fibrin formation and focused rhFIIa activity on conversion of fibrinogen to fibrin.

We were able to decrease the fibrinogen content of rhFS (9 mg rhFI/mL) relative to pdhFS (>50 mg pdFI/mL), while achieving the same *in vitro* clotting speed and strength. A typical dose of pdhFS applied to the stellate liver laceration model contained >340 mg of pdFI, whereas the typical dose of rhFS contained 90 mg of rhFI. The rhFS contained 3.6 mg per dose of rFXIIIa, whereas pdhFS contained a constitutive level of <1 mg FXIII. Importantly, when the rhFS and pdhFS were studied in the porcine hepatic wedge resection model, the rhFS had equivalent or greater hemostatic efficacy than commercial pdhFS, despite the fibrinogen concentration in the rhFS being only one-fifth that in the pdhFS.

The hemostatic potential of FS has been shown to be dependent on the degree of cross-linking achieved by FXIII [21]. Prior studies reported that depletion of FXIII in a commercial FS (Beriplast P; Aventis Behring, Marburg, Germany) decreased the ability of that sealant to control hemorrhage in porcine vascular procedures [31] and that the relative hemostatic efficacy of FS was dependent on the concentration of FXIII [32]. These previous data suggested that the optimal FXIII concentration in pdhFS was in the range of 40—80 U/mL when the pdFI concentration was 50—100 mg/mL. The data from the present study suggested that the use of rFXIIIa could produce an rhFS that had equivalent or better hemostatic properties than the pdhFS, even when the fibrinogen content of the former was relatively low.

For posttranslationally complex proteins, the signature of the host cells on the recombinant human protein potentially can cause immunologic and biologic activity issues. In the case of rhFI, the N-linked glycosylation added to the human protein sequence of the recombinant fibrinogen was the only notable posttranslational difference between pdhFS and rhFS [23]. We have seen no issues with the biologic activity of any of the recombinant proteins used in the rhFS. Definitive studies of immunologic effects would require human clinical trials.

We did not intend our hepatic wedge excision model to be a surrogate for more lethal models of traumatic hemorrhage [18], and we have not implied that rhFS or any other sealant might be useful as a stand-alone treatment for severe traumatic hemorrhage. The wedge excision model developed for this study was intended to quantify and compare the hemostatic efficacy of sealants acting without any other adjunct (such as manual compression) at the wound surface. We wanted to minimize variables that might have influenced or confounded the hemostatic activity of a sealant acting alone. We also did not intend to analyze the tissue fixation (welding or "glue") properties of the sealants, for example, for skin

grafting [33], reinforcement of gastrointestinal anastomosis [34], or sutureless anchorage of prosthetic mesh [35].

We previously estimated that it would require approximately 300 transgenic cows to produce 1 metric ton of purified rhFI per year [23]. An abundant source of rhFS might lead to increased innovation in traumatic hemostasis, acute wound stabilization, hernia surgery, and other biomaterial fields. In addition, an abundant source of recombinant fibrinogen for intravenous administration also could impact the treatment of hypofibrinogenemic conditions, such as the dilutional/consumptional state that can occur during resuscitation from traumatic hemorrhage [36]. Further studies on this condition using a hypothermic, hemodiluted swine model [12] are planned.

Acknowledgment

Appendix. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jss.2013.09.039.

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PLOS ONE

Development of a Fatal Noncompressible Truncal Hemorrhage Model with Combined Hepatic and Portal Venous Injury in Normothermic Normovolemic Swine

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Abstract

Noncompressible truncal hemorrhage and brain injury currently account for most early mortality of warfighters on the battlefield. There is no effective treatment for noncompressible truncal hemorrhage, other than rapid evacuation to a surgical facility. The availability of an effective field treatment for noncompressible truncal hemorrhage could increase the number of warfighters salvaged from this frequently-lethal scenario. Our intent was to develop a porcine model of noncompressible truncal hemorrhage with a ~50% one-hour mortality so that we could develop new treatments for this difficult problem. Normovolemic normothermic domestic swine (barrows, 3 months old, 34–36 kg) underwent one of three injury types through a midline incision: 1) central stellate injury (N = 6); 2) excision of a portal vein branch distal to the main PV trunk (N = 6); or 3) hemi-transection of the left lateral lobe of the liver at its base (N = 10). The one-hour mortality of these injuries was 0, 82, and 40%, respectively; the final mean arterial pressure was 65, 24, and 30 mm Hg, respectively; and the final hemoglobin was 8.3, 2.3, and 3.6 g/dL, respectively. Hemi-transection of the left lateral lobe of the liver appeared to target our desired mortality rate better than the other injury mechanisms.

Introduction

The two major causes of early mortality during modern military operations have been (1) hemorrhage and (2) traumatic brain injury [1,2,3,4]. It has been estimated that >80% of battlefield deaths in the modern era are secondary to uncontrolled hemorrhage [3,5,6,7,8]; moreover, recognition of vascular injury during the U.S. conflicts in Iraq and Afghanistan occurred with five-fold greater frequency than in earlier wars [9]. About half of these hemorrhagic battlefield deaths involved noncompressible torso injury or bleeding from an area not conducive to tourniquet control [2,6,10,11]. In battlefield fatalities classified as potentially survivable, noncompressible truncal hemorrhage was the cause of death in ~50% [3,8].

Most cases of compressible hemorrhage (e.g., bleeding from extremity trauma) can be controlled in the field with currently-available methods (e.g., direct compression and/or tourniquet), allowing for safe transport to a forward surgical care unit [12]. No effective field therapy currently exists, however, for hemorrhage from a noncompressible truncal injury [8,13,14]. So in order to have a chance at survival, a warfighter with a bleeding truncal injury requires immediate evacuation to a forward surgical care unit for emergency operative intervention. In modern warfare with extremely mobile combat elements, however, the time required for transport of an injured combatant to a forward surgical unit may extend to several hours [15]. Given the rapid clinical course of uncontrolled truncal hemorrhage, development of a field therapy which could be administered by a first-responder within minutes of wounding might improve survival from torso injury [3,8,14].

Most studies that have focused on the development of advanced hemostatic devices have utilized porcine models of compressible injury at various sites (e.g., aorta, femoral vessels, and liver) [13,16,17]. A few reports describing noncompressible intraabdominal hemorrhage models intended for development of local treatment have been published, including rat [18], rabbit [19] and, most recently, one model in swine [13,20,21,22]. In order to study site-directed (i.e., intraabdominal) therapies for noncompressible truncal hemorrhage, we have developed our own model of noncompressible truncal hemorrhage involving combined hepatovenous and portovenous injury in resuscitated domestic swine. In the present report we describe and characterize this porcine model of noncompressible truncal hemorrhage, compare it to

other models, and discuss how it may be utilized to develop novel field therapies for noncompressible intraabdominal bleeding.

Materials and Methods

Animal Welfare

This animal research study was carried out in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (8th ed.) from the National Research Council and the National Institutes of Health [23], and also in accordance with the Animal Welfare Act of the United States (U.S. Code 7, Sections 2131 – 2159) [24]. The animal protocol was approved by the Institutional Animal Care and Use Committee of the VA Nebraska-Western Iowa Health Care System (protocol number 00760), by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center (protocol number 11-064-07-ET), and by the Animal Care and Use Review Office of the United States Army Medical Research and Materiel Command (award number W81XWH-11-1-0836). All procedures were performed in animal facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC; www.aaalac.org) and by the Office of Laboratory Animal Welfare of the Public Health Service (http://grants.nih.gov/grants/olaw/olaw.htm). All surgical procedures were performed under isoflurane anesthesia, and all efforts were made to minimize suffering. Euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals [25]. In addition, this study was conceived, designed, documented, analyzed, and reported per the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines [26]; the ARRIVE Guidelines Checklist is shown in Table S1 in file S2.

Determination of Subject Numbers, Study Design, and Randomization

The minimum number of swine (n = 6) utilized in each group in the development of a noncompressible hemorrhage model was determined with a statistical power analysis [27] using Δ/σ (Cohen's d, in which Δ is the desired difference in means set by the observer, and σ is the estimated standard deviation) = 2.0, false positive rate (α) = 0.05, false negative rate (β) = 0.2, and power $(1-\beta)$ = 0.8. Due to the heuristic nature of the studies in this report, the animal subjects were not randomized. The intent of this study was to explore the effect of various injury mechanisms in normothermic normovolemic swine in order to obtain a reasonable model of noncompressible truncal hemorrhage. A specific injury was tested in pre-determined minimum number of subjects (six; see Methods); if that injury did not generate a reasonable outcome (i.e., a ~50% one-hour mortality), then experimentation proceeded to another injury type. Although it may have been theoretically possible to randomize subjects among the injury mechanisms tested, such a randomization would have assumed at the outset that at least one mechanism was going to have the desired mortality rate; otherwise the study goal would not have been met. Since we could not rely on this assumption during the initial planning of this study, we decided to proceed with an iterative explorative study design in order to discover an appropriate injury mechanism.

Animal Preparation

Domestic swine (castrated males, age 3 months) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska–Lincoln. Subjects underwent an acclimatization period of at least four days, during which time they underwent veterinary examination and daily observation to confirm good health. Subjects were fed ad lib with corn-soybean meal supplemented with vitamins, and maintained in SPF (specific-pathogen free) conditions. Each subject was fasted for 12 hours before the surgical procedure, but with free access to water.

Animal preparation followed a previous description [28]. Each subject was premedicated with a single 3 mL IM injection containing 150 mg Telazol (tiletamine hydrochloride and zolazepam hydrochloride, 1:1 by weight; Fort Dodge Animal Health, New York, NY), 90 mg ketamine, and 90 mg xylazine (drugs were combined in saline immediately prior to injection). After premedication each subject was weighed, and then intravenous line was established in an auricular vein. Oral endotracheal intubation (7.5 mm internal diameter cuffed tube) was performed, and anesthesia was maintained with 0.5–1.5% isoflurane using a Matrx Model 3000 Veterinary Anesthesia Ventilator (Midmark Corp., Versailles, OH). Mechanical ventilation was maintained at 12–15 breaths per minute, with a tidal volume of 10–15 mL/kg, in order to keep the end-tidal pCO_2 at 30–40 mm Hg. A heating pad was placed under each subject to support body temperature. A cutdown in the right neck (along the medial edge of the sternocleidomastoid muscle) was performed, and then a carotid arterial catheter (20 gauge) was inserted for pressure monitoring and blood sampling, followed by a jugular venous catheter (16 gauge) for isotonic fluid administration. Arterial pressure, end-tidal pCO_2, rectal temperature, cardiac electrical activity, and pulse oximetry (tongue probe) were continuously recorded with a Bionet BM5 Veterinary Monitor (Bionet America, Inc.; Tustin, CA) interfaced to a laptop computer. Each swine subject was maintained under an appropriate level of isoflurane anesthesia (indicated by absence of the corneal reflex) for the duration of the procedure; prior to euthanasia, the isoflurane was increased (see below).

Upon placement of the arterial line, 20 mL of blood was withdrawn for a serum test set, which included a complete blood count (CBC), protime (PT), partial thromboplastin time (PTT), international normalized ratio (INR), fibrinogen, arterial blood gas analysis (ABG), and thromboelastography (TEG). After completion of the above preparations, a ventral midline laparotomy incision was made through the linea alba, starting at the xiphoid process and extending inferiorly. Just superior to the urethral meatus, this incision was angled to the right in order to avoid the midline penis and urethra (but medial to the nipple line), and then was continued inferiorly down to but not into the right groin. The incision was performed with cautery to control any bleeding points from the musculoaponeurotic layers. Splenectomy then was performed, followed by placement of a cystostomy tube (18 French Foley) in the dome of the urinary bladder, secured with a purse string silk suture. The cystostomy tube exited through a stab incision in the left lower quadrant, and was connected to gravity drainage. The excised spleen was weighed, and then a volume of warm Lactated Ringers (LR; 37°C) solution equivalent to three-fold the splenic weight was administered through the jugular line, using a rapid infusion pump (Cole-Palmer Masterflex L/S; Vernon Hills, IL) set at 150 mL/min. An improvised intraabdominal pressure (IAP) monitor (100 mL IV bag) then was placed along the left paracolic gutter; the pressure line of this monitor exited out of the superior end of the laparotomy incision, and was connected to the Bionet monitor (kept level with the subject) for continuous recording of IAP. The IAP monitor was zeroed while the abdominal incision was open.

Prior to injury, any blood loss incurred during the preparation was quantified by weighing tared surgical sponges that were used to absorb lost blood, and then a volume of LR equivalent to three-

fold the pre-injury blood loss (typically <50 mL) was given using the infusion pump. Immediate pre-injury vital signs were recorded, the lower half of the midline incision was closed with towel clips, and then one of three primary injury mechanisms was applied as described below.

Injury Mechanisms

All injuries were performed in normothermic normovolemic (resuscitated) swine; only one injury was performed per subject. Immediately after injury, the abdominal incision was closed rapidly with towel clips in all subjects (Figure S1 in file S1). No post-injury treatment (other than fluid resuscitation; see below) was administered.

1. Central Liver Injury (CLI). The porcine normothermic normovolemic stellate liver laceration model was adapted from previous descriptions [28,29]. A liver laceration was created with a custom-built liver injury clamp [28], which consisted of metal tines in an X-configuration (5 cm diameter) on one arm of the clamp, and a base plate on the other arm onto which the tines seated. The base plate was placed on the inferior surface of the liver against the quadrate lobe, between the cystic duct and the portal vein. The tines were positioned over the liver dome, directly anterior (within 1 cm) to the vena cava at the base of the left medial hepatic segment. The clamp then was closed, forcing the tines through the liver dome and onto the base plate. The clamp immediately was re-opened, moved 2–3 cm to the right, and then closed again, such that the second clamp strike overlapped ~50% with the first strike.

2. Portal Vein Resection (PVR). In this injury, the left main branch of the portal vein was resected. The inferior surface of the liver was exposed by lifting the right medial and left medial lobes superiorly. The position of the left main branch of the portal vein was superficial and readily visible, running in the fissure between the left medial and left lateral lobes of the liver. Using sharp dissection and a right angle clamp, the left main branch of the portal vein was encircled and then controlled with a silk ligature. The silk ligature was placed on anterior traction, and a ~2 cm segment of the left main branch then was excised with 2–3 rapid cuts of a short curved Mayo scissors. The portal vein branch was transected just proximal to the silk ligature, the cutting then continued in the plane beneath the vein, and the excision was completed where the left main branch of the portal vein split into the left medial and left lateral lobes of the liver (Figure 1 and Figure S2 in file S1).

3. Hepatic Left Lower Lobe Hemitransection (LLLH). Hemitransection of the left lateral lobe of the liver was performed by first elevating this lobe anteriorly into the operative field (Figure 2). The base of the left lateral lobe was identified with gentle finger pinching, and then confirmed visually. A short curved Mayo scissors then was placed across the base of the left lower lobe, directed posteriorly, but taking care not to incorporate any of the left medial lobe. A single cut across the base of the left lower lobe of the liver was performed using the full length of the scissors' blade (~4 cm); see Figures 1 and 2. The liver lobe then was allowed to drop back into the abdominal cavity.

Post-injury Management

As mentioned above, the remainder (upper half) of the laparotomy incision was closed immediately after the liver injury with towel clips, which required <30 s. This produced a completely closed abdomen after injury creation. No attempt at hemorrhage control (compression, bandage application, vessel clamping, etc.) was performed for any of the injuries in this report. All injuries were intended to be negative (i.e., no-treatment)
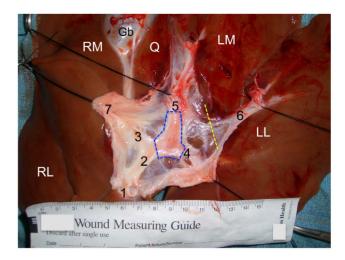
Figure 1. Dissection demonstrating the anatomy of the porcine intrahepatic portal venous system. *Ex vivo* porcine liver, inferior aspect (scale in cm). The soft tissues overlying the portal venous system have been dissected and retracted with silk stay sutures. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; Gb = gallbladder; 1 = cut orifice of main portal vein; 2 = intrahepatic portal vein; 3 = RM lobe portal vein branch; 4 = 1st LL lobe portal vein branch; 5 = cut orifice of 2nd LL lobe portal vein branch (proximal end); 6 = distal end of structure 5; 7 = pedicle containing the common bile duct and hepatic artery (reflected laterally by stitch). In this dissection the 2nd LL lobe portal vein branch was transected (the two ends are labeled as 5 and 6). The hepatic veins were not exposed in this dissection. The dashed blue polygon indicates the portion of the portal vein that was resected for the PVR injury mechanism. The dashed yellow line indicates where the cut was made across base of LL lobe for the LLLH injury mechanism. Scale = cm. [201 words].
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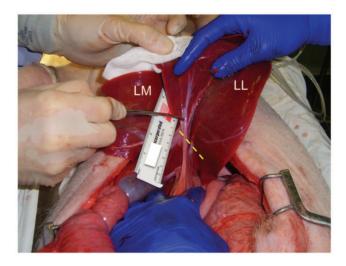
Figure 2. Operative set-up for the porcine noncompressible hemorrhage model. View of the open abdomen in a living anesthetized pig, looking toward the head. The left medial (LM) and left lateral (LL) lobes of the liver have been exteriorized through a ventral midline incision. The dashed yellow line indicates the location of the imminent cut across base of LL lobe. The tips of the scissors are touching the 2nd LL lobe portal vein branch (refer to Figure 1).
doi:10.1371/journal.pone.0108293.g002

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controls in order to document the natural course of hemorrhage from a noncompressible truncal injury.

The planned post-injury observation period was 60 min. When the mean arterial pressure (MAP) dropped below the resuscitation target pressure (defined as 80% of the pre-injury MAP), resuscitation was initiated with rapid infusion (150 mL/min) of warm LR (one liter bags stored in a 37°C incubator) into the jugular venous line. This resuscitation was maintained as long as the MAP was below the target, but was limited to 100 mL/kg (e.g., 3.5 L for a 35 kg pig). Isoflurane was maintained at 0.5–1%, as long as the subject did not demonstrate any signs of wakefulness (such as twitching or >1 spontaneous breaths between ventilator cycles).

Euthanasia

If the subject survived the 60 min post-injury observation period, then the isoflurane was increased to 5%, the incision was opened, and all procedural blood loss was evacuated from the abdomen. After 5% isoflurane had been administered for 5 min, a transverse incision was made in the diaphragm, widely opening both pleural spaces, and the supradiaphragmatic inferior vena cava was transected. Such exsanguination under anesthesia is an AVMA-approved method of euthanasia [25]. If the subject did not survive the 60 min observation period, then the above diaphragmatic incision and caval transection were performed after all procedural blood loss was evacuated from the abdomen.

Endpoints

Heart rate, MAP, pulse oximetry, end-tidal pCO_2, rectal temperature were continuously recorded (see Figure S3 in file S1), as described above. Continuous recording of intraabdominal pressure was incorporated into the experimental protocol toward the end of the study, on subjects injured with LLLH. A second serum test set was drawn 15 min after injury; a third test set was drawn at 60 min or just prior to expiration, whichever occurred first. Death was defined as MAP ≤10 mm Hg, no identifiable pressure wave on the monitor's arterial tracing, and end-tidal $pCO_2 < 5$ mm Hg. Immediately after the 60 min observation period or after the animal expired (whichever came first), the laparotomy incision was re-opened, and all clots and blood were rapidly evacuated into tared buckets with a combination of tared laparotomy pads, suction, and manual extraction. The buckets were weighed in order to calculate blood loss. Gross necropsy then was performed, including removal of the liver for inspection, dissection, photography, and documentation of injury anatomy. In the selection process of an appropriate injury for a noncompressible truncal hemorrhage model, a target of 50% one-hour mortality was chosen empirically, according the to rationale outlined in the Introduction.

Laboratory Testing

The CBC, PT/PTT, INR, fibrinogen, and ABG testing were contracted to the Clinical Laboratory of the VA Nebraska-Western Iowa Health Care System. This laboratory used the quantitative fibrinogen assay based on the von Clauss method [30]. Thromboelastography was performed with a TEG 5000 Thrombelastograph (Haemonetics Corp.; Braintree, MA) as previously described [28,31],with some modifications. Whole blood (340 μl) was incubated at 37°C and mixed with 200 mM $CaCl_2$ (20 μl). The thromboelastograph was calibrated each day of use. Each time point of each analysis was run in triplicate. TEG Analytical Software (version 4.2.2) was used to calculate the time to clot initiation (R), time to clot firmness of 20 mm (K), alpha angle (α), maximal clot strength (MA, which was directly related to the shear elastic modulus strength, G), and percent lysis 60 minutes after MA (LY60) [32].

Statistical Analysis

Numerical data is reported as the mean ± standard deviation (SD). Groups of numerical data were compared with the nonparametric Kruskal-Wallis analysis of variance. Groups of categorical data were compared with the Fisher exact test. Significance was defined as $p < 0.05$.

Results

A total of 23 swine underwent injury in this study in the following order: CLI, N = 6; PVR, N = 6; splenic pedicle transection, N = 1; and LLLH, N = 10. The first injury mechanism to be evaluated for the noncompressible hemorrhage model was CLI, which involved a stellate laceration through the dome of the liver immediately anterior to the inferior vena cava (Figure S4 in file S1). This injury mechanism has been utilized in porcine hemorrhage models since the 1990's [29]. The severity of the hemorrhage from this injury appears to be dependent on laceration of at least two major hepatic veins as they enter the intrahepatic vena cava [29]; refer to Figure S5 in file S1. In order to ensure that at least two major hepatic veins were cut, two overlapping strikes of the injury clamp were applied directly anterior to where the inferior vena cava enters the liver (see Methods). Incidentally, the towel clip incisional closure utilized in the experiments of this report could withstand a sustained intraabdominal insufflation pressure of 70 mm Hg without leakage of gas or fluid (Figure S1 in file S1).

CLI was performed on six consecutive subjects as described above, and the 60 min mortality was zero (Figure 3 and Table 1). Most of the subjects survived easily, with an average MAP of 65 mm Hg at 1 h after injury (Figure 4 and Table 1). Mean blood loss after CLI was ~1 L; the hemoglobin dropped ~4 g/dL, the platelet count decreased ~100 K, the fibrinogen level decreased by ~50%, and the base excess dropped from ~2 to about zero mmol/L. There was no significant effect on other parameters or laboratory tests (Figure 5, Figure S6 in file S1, and Tables S2, S3, and S4 in file S2). At necropsy, it was found that the injury site had sealed against the diaphragm in all subjects, which produced hemostasis of the untreated CLI (Figure S4 in file S1). Dissection of the explanted liver (Figure S4 in file S1) confirmed that 1–4 hepatic veins (median 3.0) were lacerated (defined as a cut involving more than half of the vessel circumference) in each subject (Table S4 in file S2). Since the one-hour mortality for the untreated CLI injury was zero after six subjects, it was concluded that the CLI mechanism in swine would not be sufficiently lethal for a noncompressible hemorrhage model.

The second injury mechanism tested was resection of the left main branch of the portal vein segment (Figures 1 and S2 in file S1). Six swine underwent such a resection, and only one was alive 1 h after injury (Figure 3). The other five subjects died from exsanguination 24–44 min after injury, with an average blood loss of 3.1 L, a nine point drop in hemoglobin, an increase in protime to ~20 s, a ~60% drop in fibrinogen, a decrease in bicarbonate from 29 to 16 mmol/L, and final MAP <30 mm Hg (Figures 4 and 5, Figure S6 in file S1, Table 1, and Table S4 in file S2). There were no significant changes in thromboelastographic parameters (Table S3 in file S2). At necropsy, all subjects had ongoing hemorrhage from the injury site. *Ex vivo* dissection of the liver (Figure S2 in file S1) confirmed excision of the left main branch of the portal vein in all six subjects. Since nearly all subjects died prior to the one-hour endpoint, it was concluded that
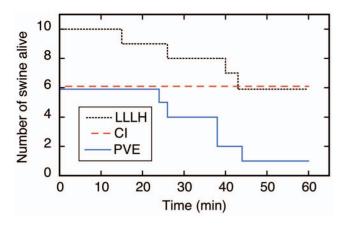
Figure 3. Kaplan Meier survival plot for the three models of noncompressible hemorrhage. Time zero = moment of injury; CLI = central liver injury (N = 6); PVR = portal vein resection (N = 6); LLLH = hepatic left lower lobe hemitransection (N = 10). doi:10.1371/journal.pone.0108293.g003

resection of the left main branch of the portal vein was too lethal for a noncompressible truncal hemorrhage model.

Incidentally, splenic pedicle transection was performed in a single subject. This was done by transecting the splenic pedicle 1 cm proximal to the mass silk ligature (securing both the splenic artery and vein) placed during the splenectomy. This subject survived the 1 h observation period easily, with only 651 mL of blood loss. At necropsy, this injury had clotted, and there was no active hemorrhage. Based on the experience with the CLI mechanism, it was decided not to pursue the splenic pedicle transection any further. This single subject is mentioned here for completeness.

The third and final injury mechanism tested for the noncompressible hemorrhage model was hemitransection of the left lateral lobe of the liver near its base, or LLLH (Figures 1, 2, and 6; also see the Video in File S3 for a demonstration of this injury mechanism). Ten subjects were injured with this mechanism, and four expired prior to the one hour endpoint (Figure 3). Continuous vital sign tracings for a typical surviving and nonsurviving subject are shown in Figure S3 in file S1. The final average MAP was 30 mm Hg (Figure 4 and Table 1); the MAP of the six surviving subjects was 39 mm Hg (Table S2 in file S2). The average blood loss for all ten subjects was 2.8 L, and the derangements in hemoglobin, BE, and INR were intermediate between the central liver injury group (minimal effect) and the portal vein resection group (excessive effect); see Figure 5, Figure S6 in file S1, Table 1, and Table S4 in file S2. There appeared to be changes in the thromboelastographs of the LLLH subjects from pre- to post-injury (Figure S7 in file S1), but the derived parameters (R, K, α, and MA) were not significantly different (Table S3 in file S2). Dissection of the explanted liver (Figure 6) demonstrated that the median number of portal vein branch and hepatic vein transections were 1.0 (range 1–2) and 1.0 (range 0–1), respectively, with seven of ten subjects demonstrating transection of one portal vein branch (the 2nd branch supplying the left lateral lobe) and one large (>7 mm) hepatic vein (draining the left lateral lobe).

The pre-injury heart rate, mean arterial pressure, rectal temperature, hemoglobin, protime, PTT, INR, fibrinogen, thromboelastography, pH, pO_2, pCO_2, and bicarbonate were not statistically different among the three injury groups (Figures 4 and 5, Figure S6 in file S1, and Tables S2 and S3 in file S2). Pre-injury platelet count, pre-injury base excess, pre-injury blood loss, and pre-injury fluid administration, were not equivalent among
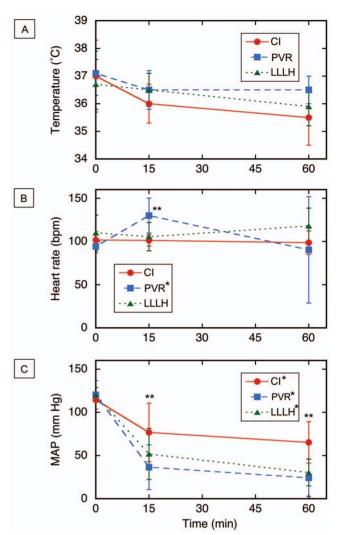
Figure 4. Vital sign data for three porcine models of noncompressible truncal hemorrhage. (A) Temperature. (B) Heart rate. (C) Mean arterial pressure (MAP). Time zero = moment of injury; CLI = central liver injury (N = 6); PVR = portal vein resection (N = 6); LLLH = hepatic left lower lobe hemitransection (N = 10). Values shown are mean ± sd; *p<0.05, Kruskal–Wallis one-way analysis of variance on all three time points for a given injury; **p<0.05, Kruskal–Wallis one-way analysis of variance on all three injuries at the indicated time point. Also refer to Table S2 in file S2. doi:10.1371/journal.pone.0108293.g004

the three injury groups (Table 2). The mean splenic mass increased ~100 g over the course of this study (Table 2), while subject weight did display significant variation (Table S4 in file S2).

Discussion

As discussed above, the reasonable outcome that was being sought in this study was a ~50% mortality within the first hour after injury. This mortality rate goal was set empirically, based upon the observations of severe noncompressible truncal hemorrhage in a military setting. Furthermore, we believed that an injury mechanism with either a higher or lower one-hour mortality probably would not allow us to discriminate differences in efficacy in subsequent comparisons of experimental treatments for noncompressible truncal hemorrhage. That is, if the injured

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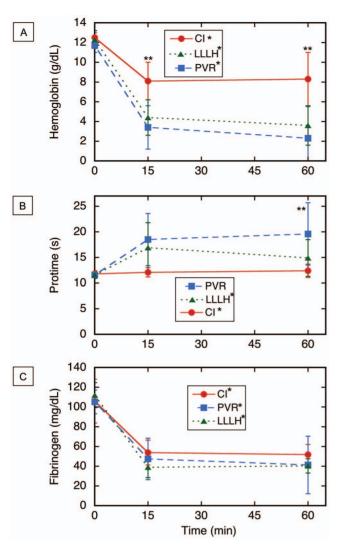
Figure 5. Hematologic testing for three porcine models of noncompressible truncal hemorrhage. (A) Serum hemoglobin. (B) Protime. (C) Serum fibrinogen. Time zero = moment of injury; CLI = central liver injury (N = 6); PVR = portal vein resection (N = 6); LLLH = hepatic left lower lobe hemitransection (N = 10). Values shown are mean ± sd; *p<0.05, Kruskal–Wallis one-way analysis of variance on all three time points for a given injury; **p<0.05, Kruskal–Wallis one-way analysis of variance on all three injuries at the indicated time point. Also refer to Table S2 in file S2.

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subject bled too "slow" or too "fast," then future comparison of treatment regimens might be meaningless.

We believe that domestic swine are a good choice to model severe hemorrhage in humans because, by the age of 3 months, domestic swine have reasonably large size (~35 kg) and blood volume (~2.6 L [33]) which makes studies of severe hemorrhage practical. We have access to an inbred population of domestic swine that has been closed for >30 years, which theoretically should reduce inter-subject variability. Furthermore, domestic swine have been used for decades to model human physiology and pathophysiology (including hemorrhage and hemostasis), and generally have produced acceptable data for these types of studies [16,17]. Although small animal models (e.g., rabbits and rodents) have produced some usable data in the field of hemostasis [18,19], information obtained from small animal models of hemorrhage
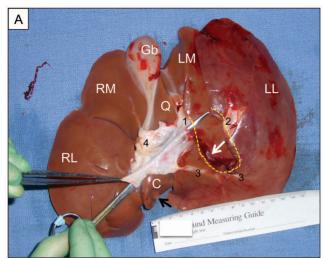
Table 1. Select endpoints (at time of death or 1 hr post-injury) for the three injury mechanisms of noncompressible hemorrhage.

Injury Type	Survival at 1 h, N (%)	Median survival time (min)	MAP, mm Hg	Blood loss, mL	Hemoglobin, g/dL	Base excess, mmol/L	Protime, s
1. CLI	6/6 (100)	68	65±24	1140±684	8.3±2.7	0.7±1.5	12.4±1.3
2. PVR	1/6 (18)	38	24±22	3142±1121	2.3±3.2	−4.9±10.5	19.6±6.1
3. LLLH	6/10 (60)	65	30±16	2853±657	3.6±2.0	−3.6±7.3	14.9±3.6
p-value	0.0025*	0.0382†	0.0192†	0.0042†	0.0084†	0.4146†	0.0298†

CLI = central liver injury; PVR = portal vein resection; LLLH = hepatic left lateral lobe hemitransection; MAP = mean arterial pressure. Continuous data expressed as mean ± standard deviation.
*Categorical data compared with the Fisher Exact Test;
†continuous data compared with the Kruskal-Wallis nonparametric analysis of variance.
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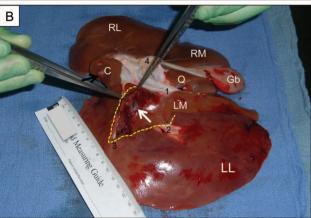
Figure 6. Postmortem liver ex vivo, demonstrating the standard injury. (A) Inferior aspect; anterior toward top of image. (B) Left inferior oblique aspect; anterior toward right of image. Scale in cm. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; C = caudate lobe (black arrow indicates location of infrahepatic IVC); Gb = gallbladder. The scissors in panel A has been inserted through the cut orifice of the main portal vein, and the scissors tip are emerging through the transected 2nd portal vein branch to the LL lobe (1); the distal end of this portal vein branch also is indicated (2). The gap in the liver parenchyma created by the injury is indicated with a dashed yellow line. The 1st portal vein branch to the LL lobe (3), visible at the bottom of the wound, was not injured in this subject. The soft tissues (including the common bile duct and hepatic artery) overlying the portal venous system have been dissected and flipped anteriorly (4). White arrow indicates orifice of transected hepatic vein to the LL lobe; the latter has a dusky appearance relative to the other lobes.
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ultimately may have limited clinical relevance because of their small organ size, blood vessel diameter, and blood volume with respect to humans.

A rabbit model of noncompressible hemorrhage was described in which partial hepatectomy (liver edge resection) was combined with a systemic administration of a Factor X inhibitor [19]. A fibrin sealant foam therapy was demonstrated to have hemostatic efficacy in this model compared to both placebo treatment or no-treatment controls. A model of noncompressible hemorrhage involving portovenous injury in domestic swine also has been described [13,20,21]. In this model, the investigators looped wires around the medial liver lobes through a midline laparotomy, and then transected these lobes by pulling the wires out of the abdomen after the incision had been closed. The subjects then were resuscitated with crystalloid with no limit on resuscitation volume. The one-hour mortality of this injury model was 90%, with a median survival time of 43 min. This group of investigators subsequently described a therapy for noncompressible hemorrhage consisting of an expansile polyurethane foam, which had demonstrable efficacy in their porcine model [20,21]. The same investigator group described another porcine model of noncompressible hemorrhage which utilized placement of a wire around the external iliac artery via a laparotomy, and subsequent transection of the artery by wire distraction after the abdominal incision had been closed [22]. One-hour mortality in this model was 78%, with a median survival time of 32 min.

Use of a "closed" abdominal technique (i.e., incision not left open) makes empiric sense in the design of a noncompressible injury model, since clinical intraabdominal hemorrhage from blunt or penetrating trauma occurs within a system that essentially is closed. Therefore, the model should be able to mimic conditions that develop in such a closed system, such as increased intraabdominal pressure, massive release/activation of clotting factors, and poor accessibility for compression-based interventions. The noncompressible model described in the present report accomplishes this goal in that a closed system is created immediately after injury by rapid closure (towel clipping) of the midline incision. The previous reports of a porcine noncompressible hemorrhage models [13,20,21,22] also accomplished this goal, as the laparotomy incision in that model was closed at the time of injury.

In comparison to the recently-published swine models of noncompressible torso hemorrhage, our model had a more moderate one-hour mortality (40%), but produced similar decreases in blood pressure and hemoglobin. The hepatovenous/portovenous injury in our model was conceptually similar to that of the wire-distraction model [13], though the technique of inducing the injury obviously was different between these two models. We also utilized routine pre-injury splenectomy (see below discussion), not done in the above studies. Our subject size was 5–10 kg smaller than used in the above studies. We limited our resuscitation volume to 100 mL/kg of crystalloid (or 3.5 L in a 35 kg subject, given at 150 mL/min, with a resuscitation target of 80% of the pre-injury MAP). The resuscitation fluid limit in the other described porcine portovenous noncompressible injury model was 10 L (given at 100 mL/min) with a resuscitation target MAP of 65 mm Hg [13,20,21]; in this group's description of a porcine noncompressible iliac artery injury model, however, the crystalloid resuscitation was limited to 1 L (two 500 mL boluses within the first 20 min) [22].

Of note, previous animal [34] and clinical studies [35] have suggested that in subjects with uncontrolled hemorrhage and no immediate operative intervention, hypotensive resuscitation (variably defined, but typically meaning administration of very little or no intravenous fluid) will increase survival. The concept of hypotensive resuscitation has been discussed in the literature since the early 1900's [36]. Although studies on the clinical benefits of hypotensive resuscitation have not been uniformly positive [37], the current Tactical Combat Casualty Care (TCCC) guidelines recommend the use of hypotensive resuscitation (<1 L of 6% hetastarch) in prehospital management of uncontrolled hemorrhage, and then early use of 1:1 blood products (1 unit of plasma transfused with every unit of packed red blood cells) in conjunction with hemorrhage control in the surgical unit [5,38,39,40,41]. The question of whether "low" vs. "high" volume crystalloid resuscitation will produce better survival in our porcine noncompressible

Table 2. Pre-injury parameters not equivalent among the three injury groups.

Injury Type	Pre-injury platelet count (1,000/μL)	Pre-injury base excess (mmol/L)	Pre-injury blood loss (mL)	Pre-injury fluid (mL)	Splenic Wt (g)
1. CLI	368±52	2.3±1.3	289±48	917±122	231±47
2. PVR	409±68	4.9±1.7	339±71	930±156	261±50
3. LLLH	288±61	3.5±2.6	374±37	1160±176	335±34
*p-value	**0.0134**	**0.0379**	**0.0238**	**0.0043**	**0.0014**

CLI = central liver injury; PVR = portal vein resection; LLLH = hepatic left lateral lobe hemitransection. Data expressed as mean ± standard deviation;
*Kruskal-Wallis nonparametric analysis of variance.
Pre-injury blood loss includes the full mass of the spleen plus any incidental blood loss incurred during subject preparation for injury. Pre-injury fluid = intravenous isotonic crystalloid administered prior to injury.
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model was not addressed in the present study. Regarding 1:1 blood product utilization, we and the other investigators [13,20,21,22] developing these porcine models of "battlefield" noncompressible, uncontrolled hemorrhage have not employed this type of resuscitation; some investigators, however, have infused salvaged autologous whole blood in similar models [42].

Routine splenectomy during the pre-injury preparation of porcine hemorrhage models has been a common but controversial practice [43]. It has been argued that the contractile porcine spleen can participate in an "auto-transfusion" phenomenon during severe blood loss [44], which may have a confounding effect on the subject's response to massive blood loss. While routine splenectomy is a commonly-practiced preparatory technique in porcine models of severe hemorrhage, the utilization of splenectomy is not universal in this type of research [43,45]. Obviously, a trauma victim does not undergo splenectomy with fluid replacement prior to incurring a torso injury; the practice of pre-injury splenectomy in swine hemorrhage models may introduce other effects that are not entirely understood [43]. Of note, it was not our intent with the present study to determine whether splenectomy should or should not be performed as a component of these hemorrhage models.

The meaning of the difference in the pre-injury platelet counts was not clear, as pre-injury platelet count means were all within normal limits. Similarly, the meaning of the difference in the pre-injury base excess values probably was not relevant, in that these values also were within normal limits, and none of the subjects demonstrated signs of hypovolemia or under-resuscitation prior to injury. The meaning of the difference in splenic mass among the three injury groups was not clear; this simply may have been a random event. The differences in pre-injury blood loss (which included spleen mass) and pre-injury fluid administration could be explained by the difference in splenic mass among the three injury groups (Table 2), because the splenic mass directly influenced both the pre-injury blood loss and pre-injury fluid administration (see Methods).

The primary utility of a large animal model of noncompressible intraabdominal hemorrhage is, of course, to develop treatments for this difficult clinical problem. An expansile polyurethane foam has been demonstrated to have efficacy in the wire-induced portovenous injury model of noncompressible hemorrhage [21]. This foam is nonresorbable, and was associated with brief periods of marked intraabdominal hypertension (>100 mm Hg) after injection, followed by a plateau of intraabdominal pressure at ~30 mm Hg [21]. While effective, there has been some concern for foam-induced pressure injury to the intestines in these studies [20,21]. Using the model of the present report, we currently are developing an alginate-based foam supplemented with an

optimized human fibrin sealant [28] for treatment of noncompressible hemorrhage (see Figure S8 in file S1). This foam formulation is fully-resorbable, and has not been associated with excessive intraabdominal pressure in preliminary experiments (unpublished data).

The observation of inter-group differences in some preoperative descriptors (Table 2) was unexpected. Although it could be argued that some of these differences were not relevant, the inter-group difference in splenic weight is hard to explain. It is possible that the domestic swine, which were drawn from a large, closed (inbred) population of inbred domestic swine were not as uniform as initially assumed. Alternatively, the difference in splenic weight among the three injury groups simply could be ascribed as a chance result (i.e., a false positive), which theoretically should occur once in every 20 statistical tests, if the α is set at 0.05. The actual effect of the differing splenic weights on the noncompressible hemorrhage in this study was not clear.

Conclusions

A porcine model of noncompressible torso hemorrhage was developed which involved a combined hepatic vein and portal vein transection. The model had a one hour mortality of 40%. This model should be useful for development of field therapies for uncontrolled hemorrhage after truncal hemorrhage, which currently accounts for a large proportion of battlefield deaths among warfighters. An effective therapy for uncontrolled truncal hemorrhage may reduce battlefield mortality.

Supporting Information

File S1 Figures S1–S8.
(PDF)

File S2 Tables S1–S4.
(ZIP)

File S3 Video.
(MP4)

Acknowledgments

Author Contributions

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Abstract presented at the 2014 Academic Surgical Congress, San Diego, February 2014

"Development of a porcine model of severe noncompressible truncal hemorrhage"

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Introduction
Noncompressible truncal hemorrhage and brain injury currently account for most early mortality in warfighters on the battlefield. There is no effective treatment for noncompressible truncal hemorrhage, other than rapid evacuation to a surgical facility. The availability of an effective field treatment could increase the number of warfighters salvaged from this frequently-lethal scenario. Our intent was to develop a porcine model of noncompressible truncal hemorrhage so that we could study new treatments for this clinical problem.

Methods
Normovolemic normothermic domestic swine (barrows, 3 months old, 34-36 kg) were administered isoflurane anesthesia followed by line placement, cystotomy, and splenectomy, and then underwent one of three injury types through a midline incision: 1) central stellate injury, administered with x-shaped tines applied anterior to the suprahepatic inferior vena cava (N=6); 2) excision of a portal vein (PV) branch distal to the main PV trunk (N=5); or 3) near-transection of the left lateral lobe (LLL) of the liver at its base, which cut the hepatic vein (HV) and PV to this lobe (N=10). The midline incision was towel clipped immediately after injury, and animals were monitored for 60 min or until death. Resuscitation was performed with warm LR (max volume = 100 mL/kg), with a target MAP set at 80% of pre-injury MAP.

Results
The starting weight, MAP, hemoglobin, arterial pH, INR, and pre-injury blood loss did not differ among groups ($p > 0.05$, ANOVA). Select variables after injury are summarized in the Table. One type 3 pig required only 56% of the resuscitation fluid limit, otherwise all subjects received the 100 mL/kg maximum. Death occurred at 34±8 min in the type 2 pigs. Postmortem evaluation revealed that 2-3 HVs were transected by injury type 1 (central stellate), and that 1-2 PV branches plus 1 HV were transected by injury type 3 (LLL transection).

Conclusions
A goal of this work was to develop a model which would provide hemorrhage severe enough such that differential effects of future experimental therapies could be observed, but not so severe such that rapid death would preclude the ability to see an interventional effect. Hemorrhage from the central stellate injury was inadequate, while hemorrhage from excision of a proximal branch of the PV was too severe (i.e., the subjects died too quickly). Near-transection of the hepatic LLL at its base (type 3 injury), however, appeared to yield hemorrhage of appropriate severity, as indicated by the 40% 1-hour mortality and intermediate values for MAP, blood loss, and other variables. Our plan is to use this last injury model in the development of therapies for noncompressible truncal hemorrhage.

Table. Select variables at time of death or 1 hr post-injury.

Injury Type	Survival at 1 h, N (%)**	Final MAP, mm Hg (mean±sd)*	Blood loss, mL (mean±sd)*	Final Hb, g/dL (mean±sd)*	Final BE, mmol/L (mean±sd)	Final INR (mean±sd)#
1. Central	6/6 (100)	65 ± 24	1140 ± 684	8.3 ± 2.7	0.7 ± 1.5	1.1 ± 0.1
2. Excision PV branch	0/5 (0)	16 ± 4	3581 ± 353	1.1 ± 0.7	-6.4 ± 11.0	5.2 ± 4.4
3. LLL transect	6/10 (60)	28 ± 16	2852 ± 657	3.3 ± 2.0	-3.6 ± 7.3	3.1 ± 3.7

LLL = left lateral lobe of liver; PV = portal vein; MAP = mean arterial pressure; Hb = hemoglobin; BE = base excess; INR = international normalized ratio; **$p < 0.05$, Fisher Exact Test; *$p < 0.05$, ANOVA; #$p < 0.05$, Kruskal-Wallis nonparametric analysis of variance.

Abstract Detail

10TH ANNUAL ACADEMIC SURGICAL CONGRESS

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Title   Synthetic Resorbable vs. Cellulose Bandage for Minor Hemorrhage in a Porcine Model

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Classifications

Type	Basic/Translation
Scientific Area	Wound Healing/Fibrosis
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Conflict of Interest Declarations   Off Label Use: No

Introduction: Commercially-available topical hemostats for minor hemorrhage incurred during elective surgical procedures are relatively expensive. We believe that more economical synthetic hemostats could be produced. Our objective here was to compare the efficacy and toxicity of a synthetic resorbable hemostatic bandage vs. an analogous commercial product in a porcine model of minor hemorrhage.

Methods: For the nonsurvival efficacy study, anesthetized domestic swine (boars,

Abstract

3 months, 29-40 kg) underwent arterial/venous line placement and splenectomy. A 1 x 8 cm section of liver was resected from the edge of the left lateral lobe, and test bandage (macroporous polycaprolactone mesh, PCL; N = 10) or oxidized regenerated cellulose (ORC; Surgicel®, Ethicon®; N = 10) was applied with manual pressure for 5 minutes. Resuscitation then was performed with warm LR (target MAP = 80% of preinjury), and blood loss was measured 60 min after injury. For the survival toxicity study, a similar resection technique was employed (N = 6 for each material), and necropsy was performed at 30 days to evaluate for bandage toxicity (subject growth, serum chemistry, histology).

Results: Pre-injury weight, VS, and laboratory testing did not differ among groups. Resection mortality was zero. In the efficacy study, there were no differences between the PCL vs. ORC groups in blood loss or other post-injury variables (Table), except that the resuscitation fluid volume in the ORC group was greater. Other results from the efficacy study not shown in the Table include platelet counts and coagulation testing (no significant differences). Other than minor granuloma formation at the implantation site with both PCL and ORC, the survival study did not reveal any measurable toxicity.

Conclusion: The efficacy and toxicity of the PCL test bandage vs. the ORC comparator were not different in a porcine model of minor hepatic hemorrhage. Based on projected costs of production (not shown), the PCL bandage could represent a lower-cost alternative to ORC for the treatment of minor surgical bleeding.

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Table. Data (mean ± sd) from efficacy study at the 60 min end-point

Variable	PCL	ORC	unpaired t-test
Resection mass (g)	7.6 ± 1.9	6.7 ± 2.0	0.32
Blood Loss (mL)	93 ± 27	111 ± 55	0.38
LR Resuscitation (mL)	594 ± 425	1952 ± 1363	0.01*
MAP (mm Hg)	89 ± 8	93 ± 11	0.30
Hb (g/dL)	12.8 ± 1.2	12.9 ± 0.9	0.85
Platelets (1,000/µL)	266 ± 56	327 ± 94	0.11

2014 Update on Hemostasis Project

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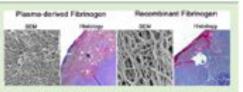
ISR, San Antonio, May 30, 2014

Recombinant Human Fibrinogen That Produces Thick Fibrin Fibers with Increased Wound Adhesion and Clot Density

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○ Supporting Information

ABSTRACT: Human fibrinogen is a biomaterial used in surgical tissue sealants, scaffolding for tissue engineering, and wound healing. Here we report on the post-translational structure and functionality of recombinant human FI (rFI) made at commodity levels in the milk of transgenic dairy cows. Relative to plasma-derived fibrinogen (pdFI), rFI predominately contained a simplified, neutral carbohydrate structure and >4-fold higher levels of the γ′-chain transcriptional variant

that has been reported to bind thrombin and Factor XIII. In spite of these differences, rFI and pdFI were kinetically similar with respect to the thrombin-catalyzed formation of protofibrils and Factor XIIIa-mediated formation of cross-linked fibrin polymer. However, electron microscopy showed rFI produced fibrin with much thicker fibers with less branching than pdFL. In vivo studies in a swine liver transection model showed that, relative to pdFI, rFI made a denser, more strongly wound-adherent fibrin clot that more rapidly established hemostasis.

1. INTRODUCTION

Known as coagulation Factor I (FI), fibrinogen is a complex protein which polymerizes to form a wound adherent fibrin barrier that stops bleeding and acts as a scaffold for healing.[1-4] During the healing process, the fibrin clot is enzymatically digested and reabsorbed.[2,6] These characteristics make FI naturally useful as a biomaterial in surgical tissue sealants[5-7] and as an important tool in tissue engineering.[8-11] For decades, FI has been used in the treatment of hemorrhage incurred on battlefields, in civilian trauma,[12] and in surgical procedures.[13] The therapeutic transplantation of tissue made from autologous cells frequently uses a provisional matrix made of cross-linked fibrin to help culture and then deliver layers of cells into debrided wound sites.[10] Because of its role in making fibrin polymer, FI is a protein present at a high concentration of 2–4 g/L in human plasma,[14] and its clinical applications typically use large 0.1–2 g doses.[5] Unfortunately, a safe and abundant supply of plasma-derived FI (pdFI) is limited worldwide by the availability of pathogen-screened plasma.[15,16] Surgical applications for tissue sealants[13] in the United States indicate that FI would need to be manufactured at amounts greater than several metric tons per annum (Supporting Information, Table S1).

FI is a transcriptionally and post-translationally complex molecule. For example, after transcription of three separate genes, the human liver translates and assembles two Aα, two

Bβ, and two γ-polypeptides into hexameric FI having a molecular weight of 340 kDa.[17,18] Assembly arises from the restrictive pooling of free chains within the endoplasmic reticulum prior to secretion as a holoprotein. In addition, there are two variations of the γ-chain. About 11% of pdFI contain a subpopulation of the γ-chain (γ′), which is a result of an alternative mRNA splicing event that replaces the four amino acid residues on the carboxy-terminal of the γ-chain with a 20 amino acid fragment.[19,20] The γ′ and γ subpopulations are both physiologically important.[20-24] The activation of FI, assembly into protofibrils and fibrin cross-linking are also affected by post-translational sulfation,[25] phosphorylation,[26] and glycosylation.[27,28] Importantly, the γ′-chain content and the post-translational modifications of FI have been associated with opposing changes in fibrin fiber diameter, porosity, and degree of branching.[27,29-31] Past reports show that the impact of increased γ′ content on fibrin formation is slowed fibrin polymerization, decreased fiber diameter with increased branching that produces smaller pores.[29-31] In contrast, in vitro deglycosylation of FI produced fibrin structure with a larger fiber diameter, decreased branching, and larger pores.

A totally recombinant human fibrin sealant

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ABSTRACT

Background: Applications of plasma-derived human fibrin sealants (pdhFS) have been limited because of cost, limited supply of pathogen-screened plasma, the need for bioengineering improvements, and regulatory issues associated with federal approval. We describe a totally recombinant human fibrin sealant (rhFS), which may engender an abundant, safe, and cost-effective supply of efficacious fibrin sealant.

Materials and methods: A first-generation rhFS made from recombinant human fibrinogen (rhFI; produced in the milk of transgenic cows), activated recombinant human factor XIII (rhFXIIIa; produced in yeast), and recombinant human thrombin (rhFIIa; purchased, made in animal cell culture) was formulated using thromboelastography (TEG). The hemostatic efficacy of rhFS versus commercial pdhFS was compared in a nonlethal porcine hepatic wedge excision model.

Results: The maximal clot strength of rhFS measured in vitro by TEG was not statistically different than that of pdhFS. TEG analysis also showed that the rhFS gained strength more quickly as reflected by a steeper α angle; however, the rhFS achieved this clot strength with a 5-fold lower factor I content than the pdhFS. When these fibrin sealants were studied in a porcine hepatic wedge excision model, the hemostatic scores of the rhFS were equivalent or better than that of the pdhFS.

Conclusions: The bioengineered rhFS had equivalent or better hemostatic efficacy than the pdhFS in a nonlethal hemorrhage model, despite the factor I concentration in the rhFS being about one-fifth that in the pdhFS. Because the rhFS is amenable to large-scale production, the rhFS has the potential to be more economical and abundant than the pdhFS, while having a decreased risk of blood-borne pathogen transmission.

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1. Introduction

The use of dried plasma as a topical hemostatic aid was documented in 1909 [1]. The combination of relatively pure fibrinogen (factor I or FI) with thrombin to make fibrin glue or foam was described in 1944 [2], but it was not until improved purification technology became available that fibrin sealants (FS) became commercially available in the 1970s [1]. Since that time, the efficacy of FS products as a topical hemostat or tissue adhesive has been demonstrated in numerous elective clinical

Part 1

Development of a noncompressible truncal hemorrhage model

PLOS ONE

Development of a fatal noncompressible truncal hemorrhage model with combined hepatic and portal venous injury in normothermic normovolemic swine
--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Full Title:	Development of a fatal noncompressible truncal hemorrhage model with combined hepatic and portal venous injury in normothermic normovolemic swine
Short Title:	Porcine model of noncompressible hemorrhage
Corresponding Author:	Mark A. Carlson, MD University of Nebraska Medical Center Omaha, NE UNITED STATES
Keywords:	Swine; noncompressible; hemorrhage; torso; truncal; Trauma; hepatic; portal; hemostasis; porcine; liver; large animal model
Abstract:	Noncompressible truncal hemorrhage and brain injury currently account for most early mortality of warfighters on the battlefield. There is no effective treatment for noncompressible truncal hemorrhage, other than rapid evacuation to a surgical facility. The availability of an effective field treatment for noncompressible truncal hemorrhage could increase the number of warfighters salvaged from this frequently-lethal scenario. Our intent was to develop a porcine model of noncompressible truncal hemorrhage with a ~50% one-hour mortality so that we could develop new treatments for this difficult problem. Normovolemic normothermic domestic swine (barrows, 3 months old, 34-36 kg) underwent one of three injury types through a midline incision: 1) central stellate injury (N = 6); 2) excision of a portal vein branch distal to the main PV trunk (N = 6); or 3) hemi-transection of the left lateral lobe of the liver at its base (N = 10). The one-hour mortality of these injuries was 0, 82, and 40%, respectively; the final mean arterial pressure was 65, 24, and 30 mm Hg, respectively; and the final hemoglobin was 8.3, 2.3, and 3.6 g/dL, respectively. Hemi-transection of the left lateral lobe of the liver appeared to target our desired mortality rate better than the other injury mechanisms
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	Michael J Duggan Massachusetts General Hospital mduggan2@partners.org Expertise
	Anthony E Pusateri US Army Medical Research and Materiel Command anthony.pusateri@amedd.army.mil Expertise
Opposed Reviewers:	
Additional Information:	

Mechanisms Tested:
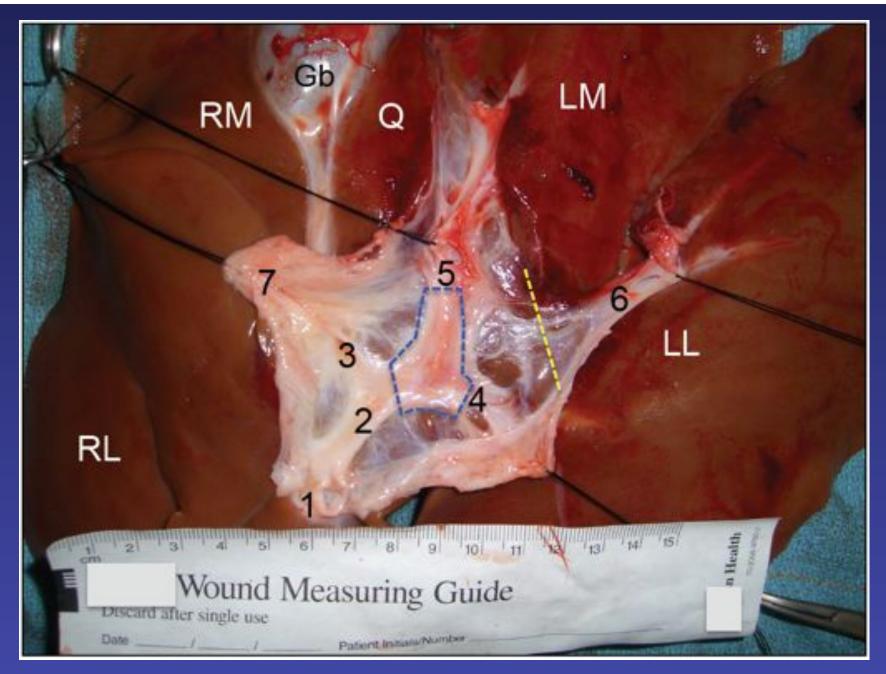
1. Central Liver Injury

2. Portal Vein Resection

3. Left Lower Lobe Hemitransection
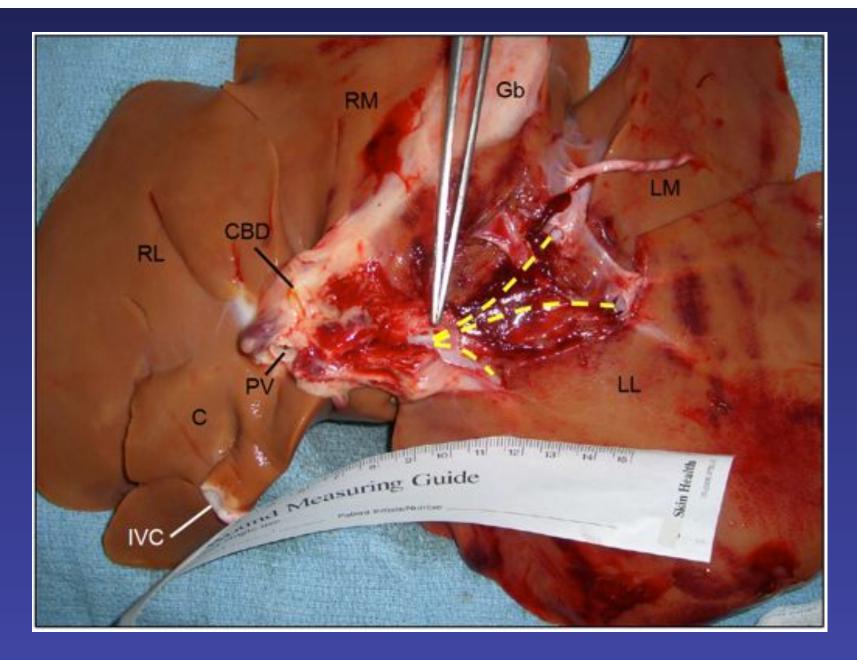
Hepatic Venous Drainage Into IVC
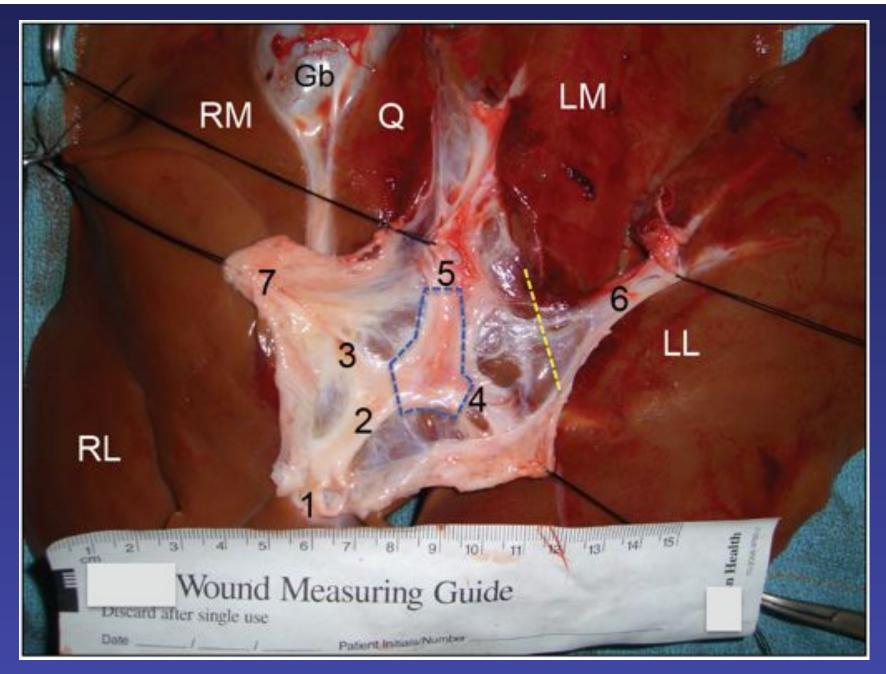
Central Liver Injury: Necropsy
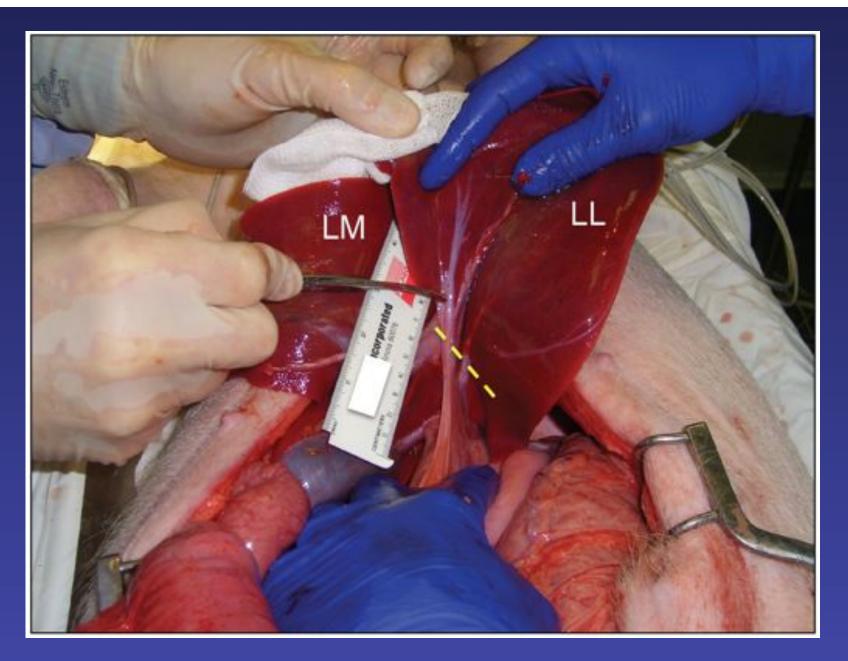
Portal vein injury mechanisms

Portal Vein Resection : Necropsy

Portal vein injury mechanisms

LM

LL

Left lateral lobe hemitransection

Left Lateral Lobe Hemitransection: Necropsy

Left Lateral Lobe Hemitransection
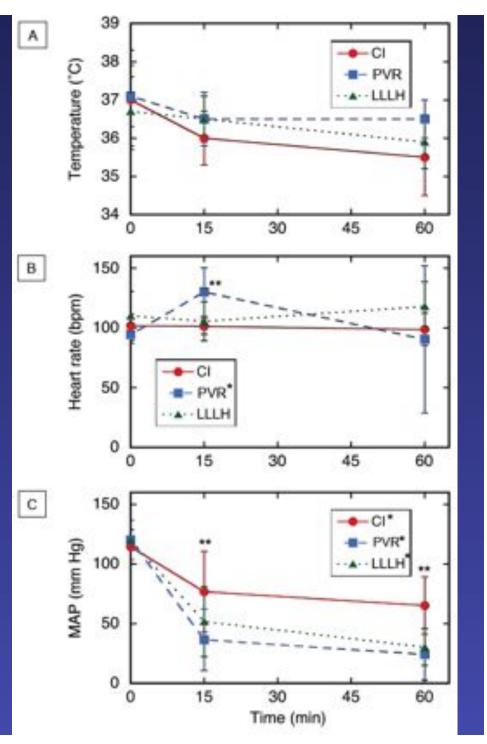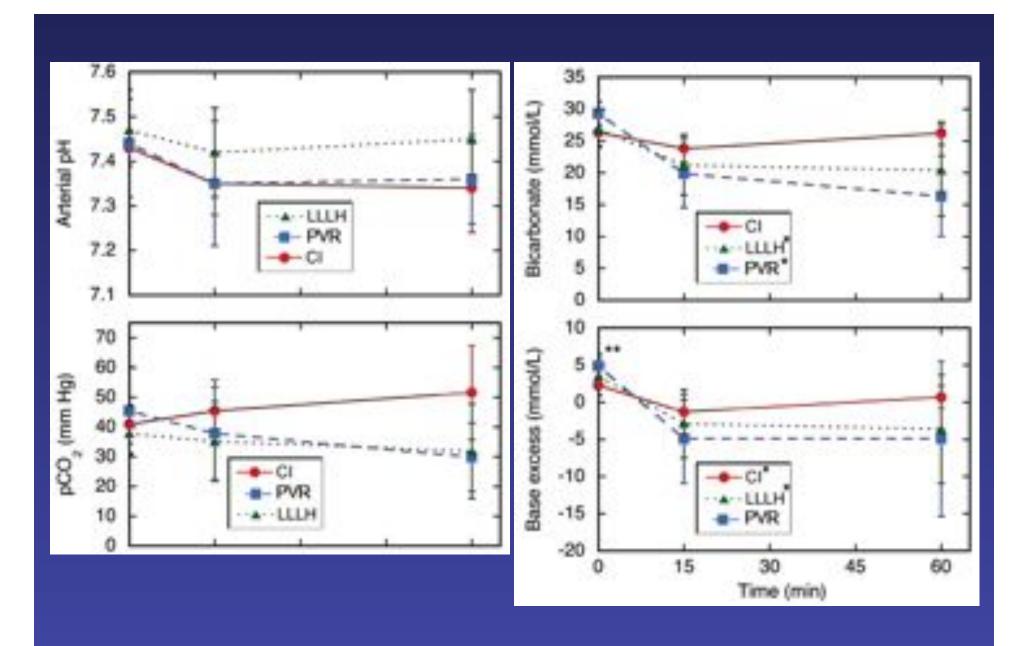
Nonsurvivor

Survivor

Labs During 1 h Post-injury Observation Period
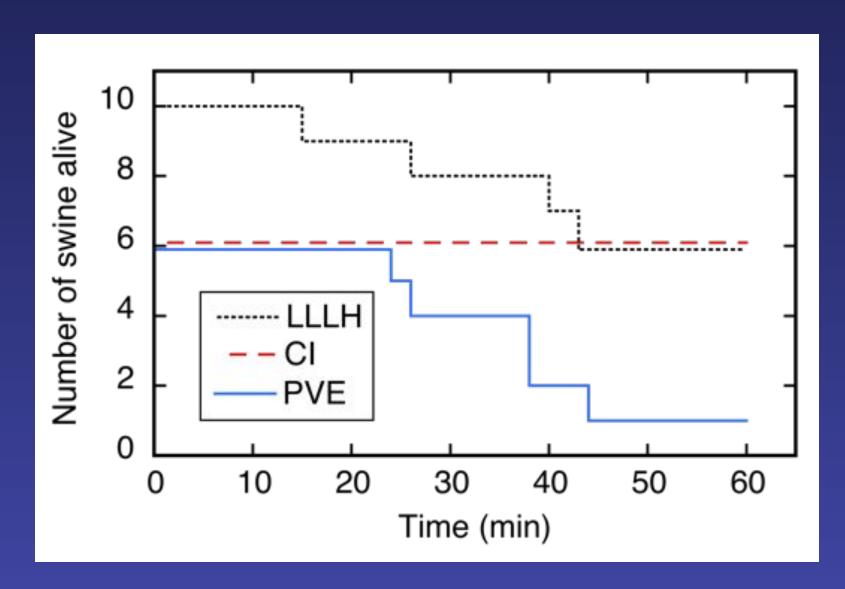
VS During 1 h Post-injury Observation Period
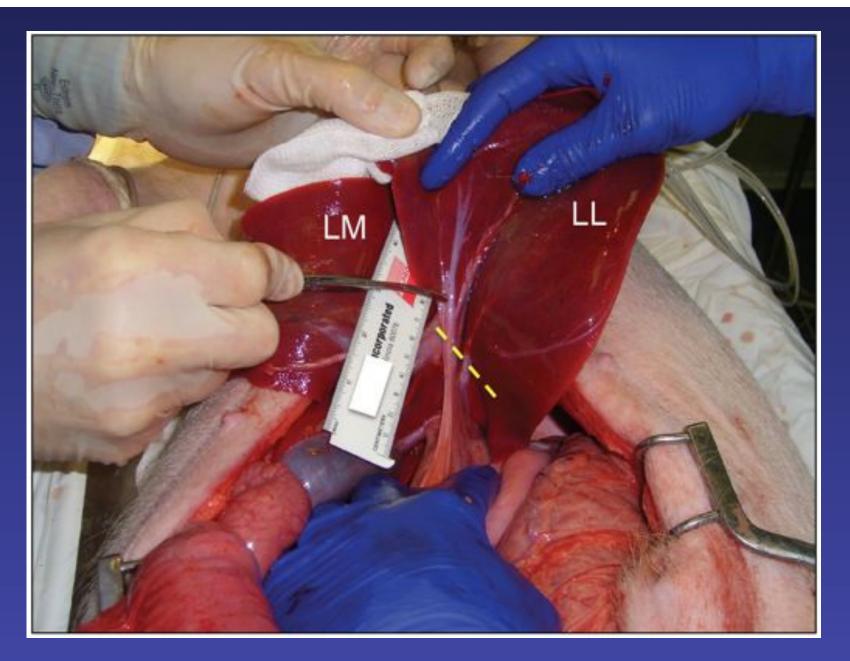
ABGs During 1 h Post-injury Observation Period
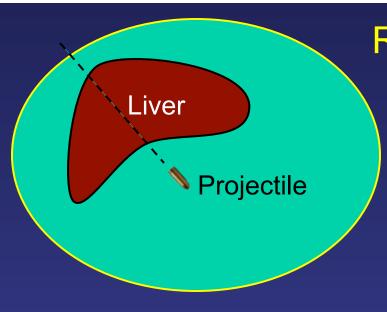
Kaplan Meier of Injury Mechanisms
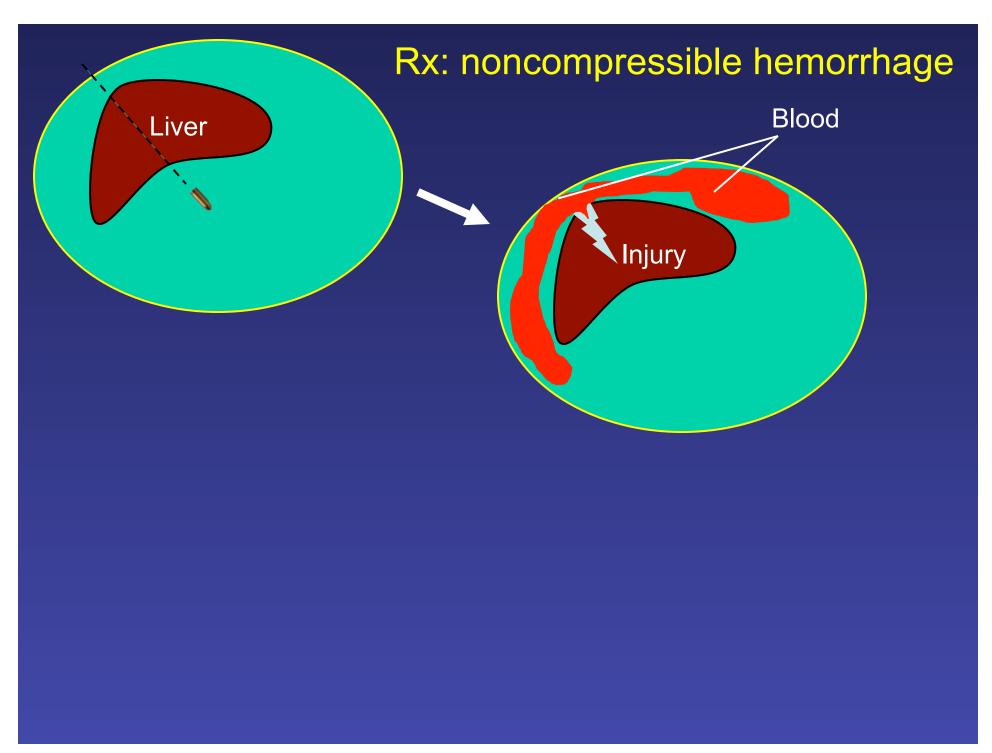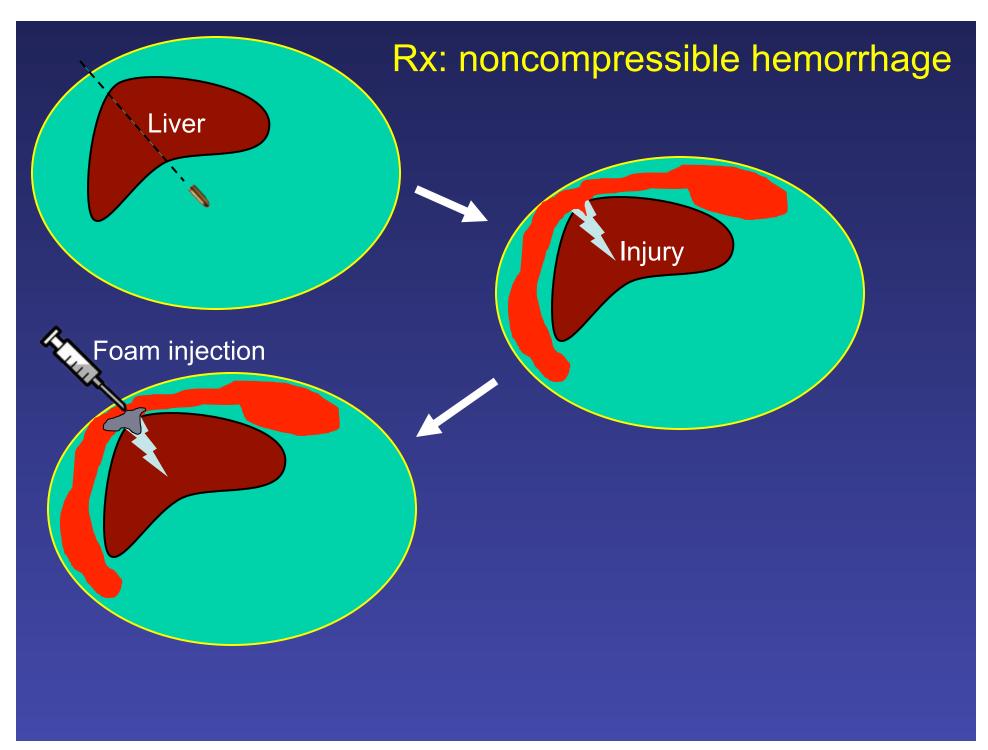
Left lateral lobe hemitransection
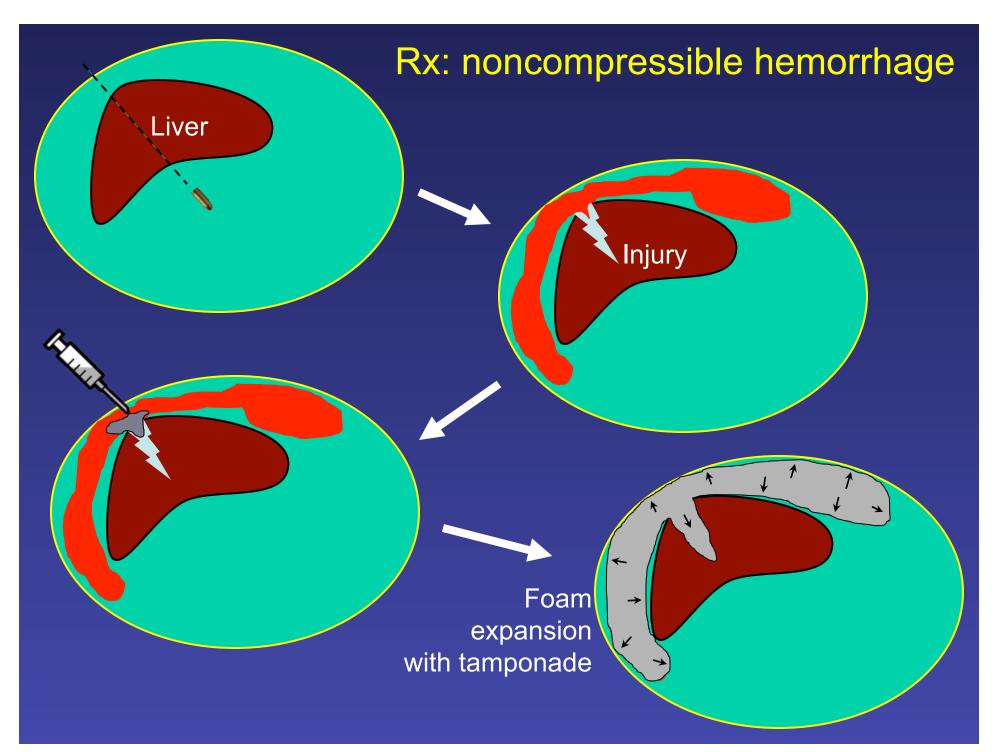
Part 2

Development of a injectable foam therapies

Rx: noncompressible hemorrhage

Liver

Projectile

Rx: noncompressible hemorrhage

Liver

Blood

Injury

Rx: noncompressible hemorrhage

Liver

Injury

Foam injection

Rx: noncompressible hemorrhage

Liver

Injury

Foam
expansion
with tamponade
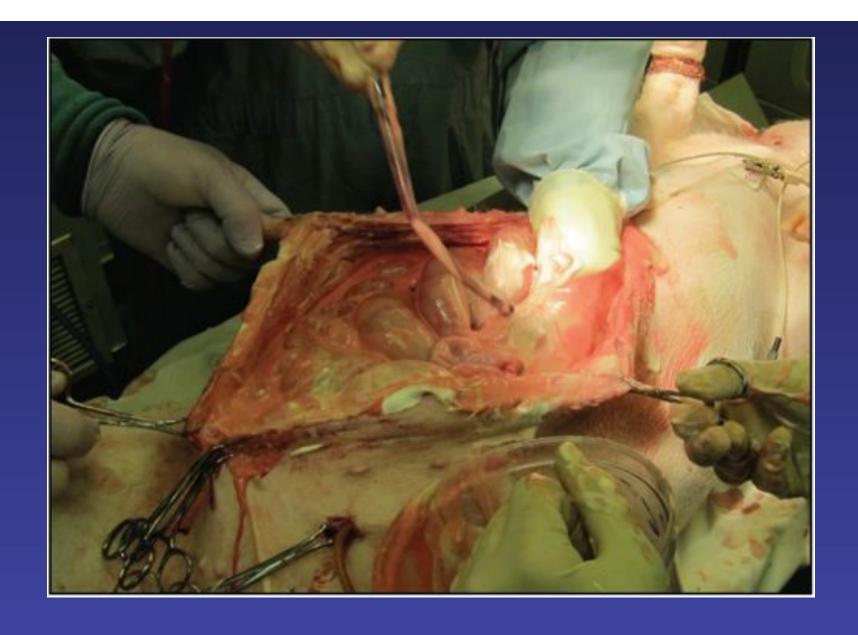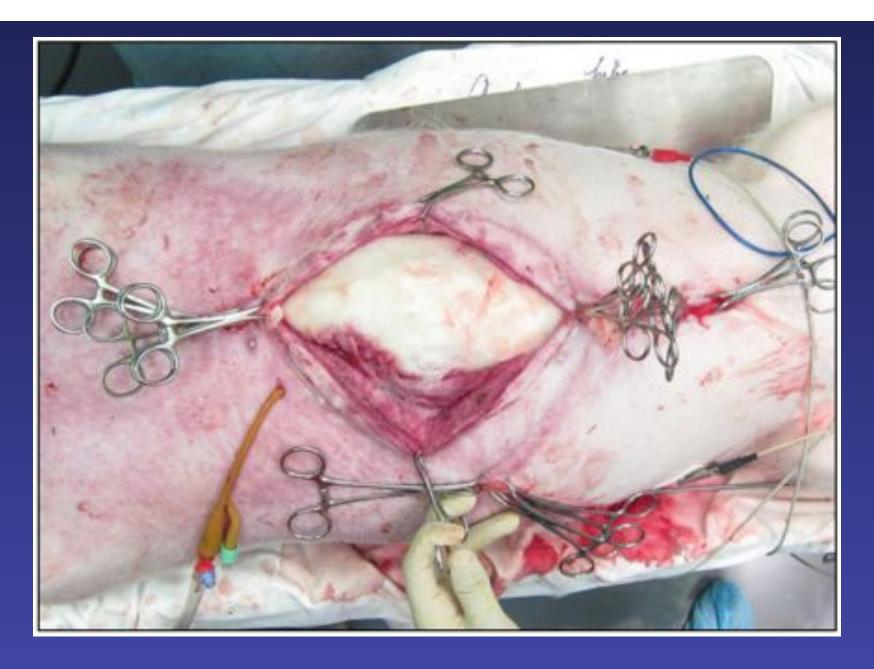
Barbasol-Based Treatment

Calcium Alginate Foam At Necropsy

Calcium Alginate Foam At Necropsy

Incision Re-opening At Necropsy

Apparatus For Injection Of Alginate + Biologics
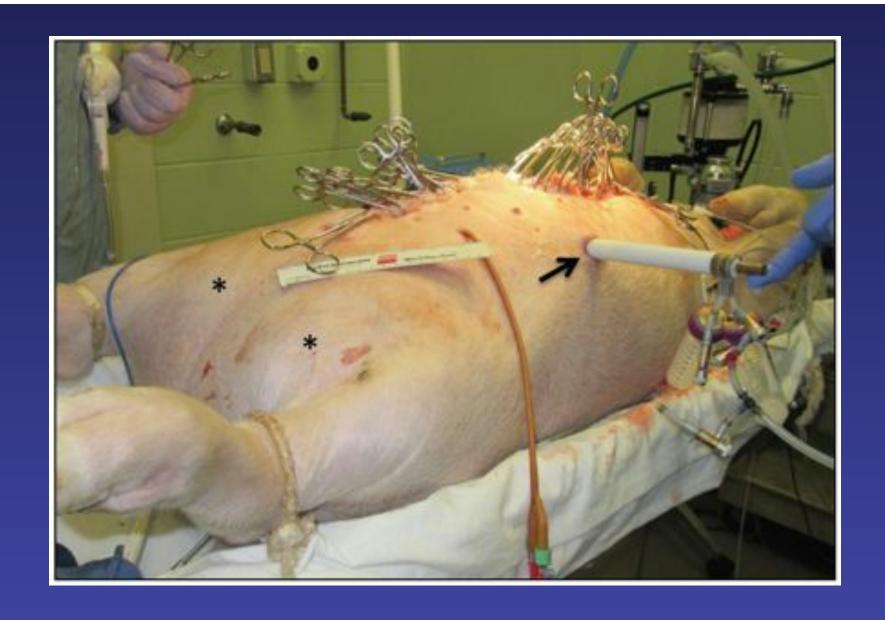
Alginate Reservoir And CaCl$_2$ Pumps

Placement Of Injector Tip Through The Incision
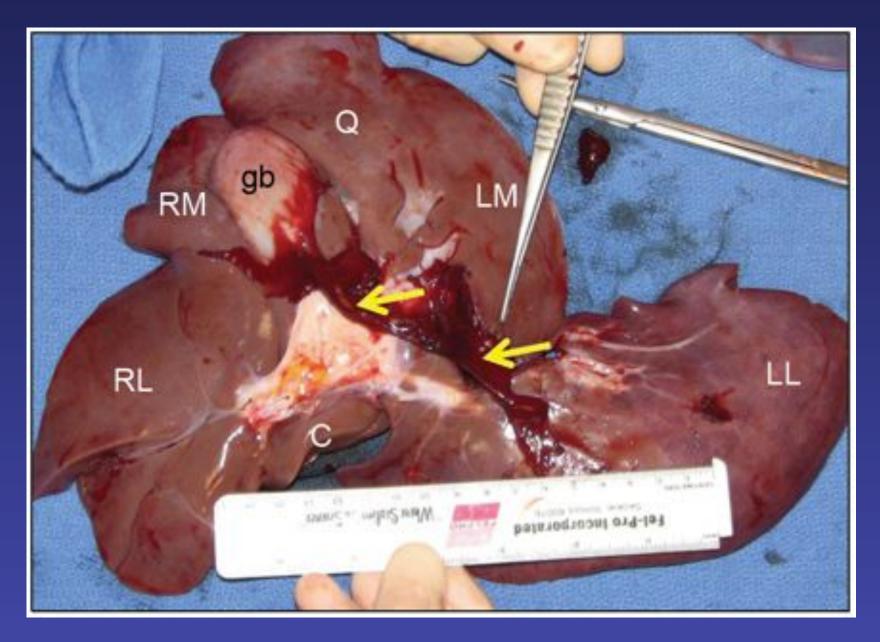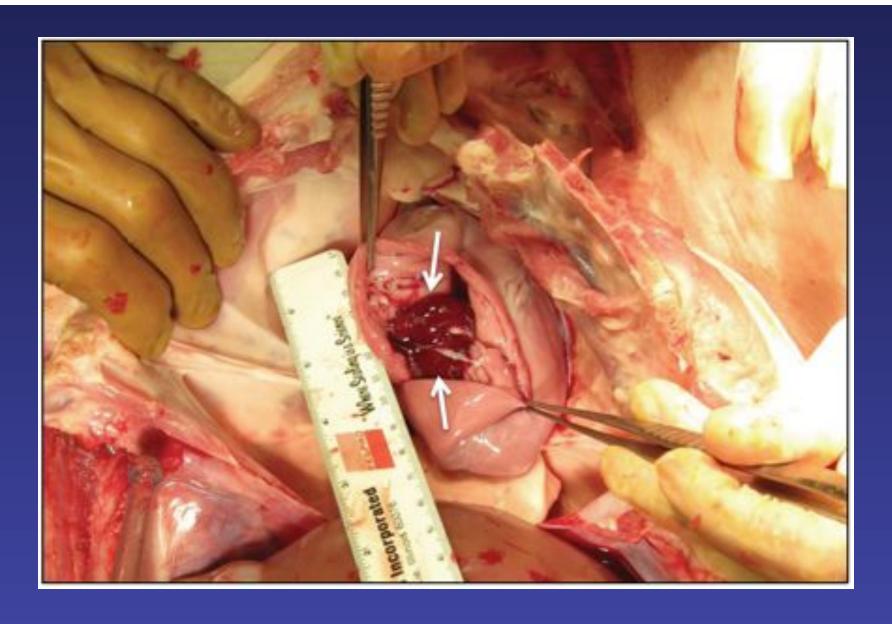
Lateral Placement Of Foam Injector Apparatus
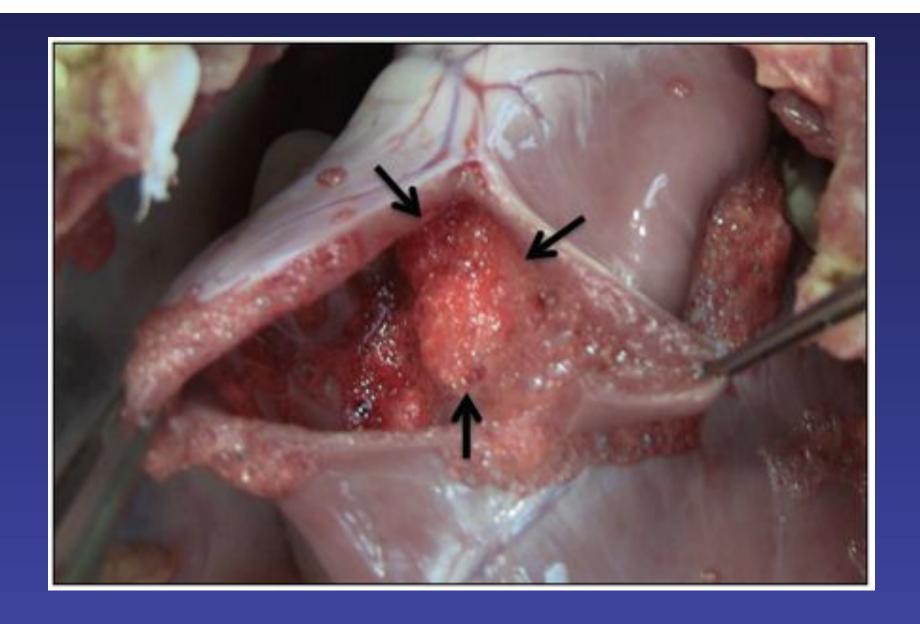
Abdominal Distention ~30 min After Treatment
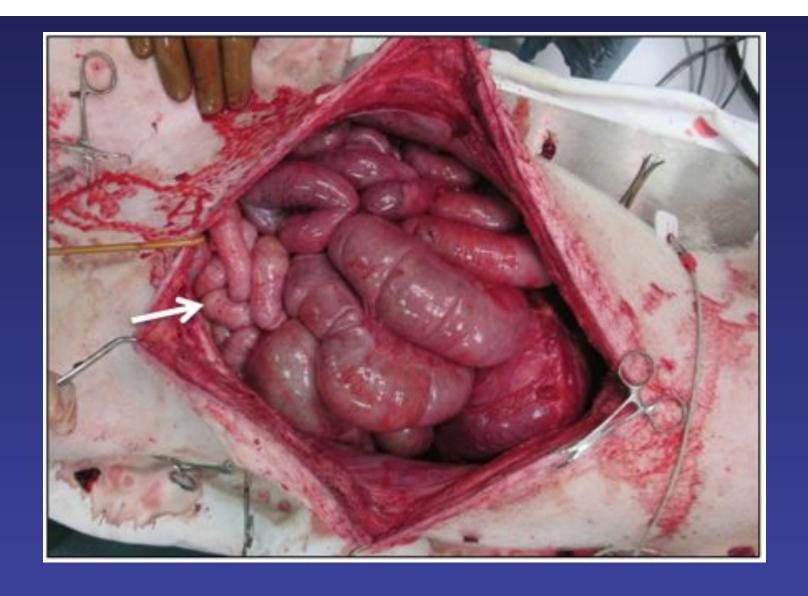
1 h Survivor, Treated With Alginate + Biologics
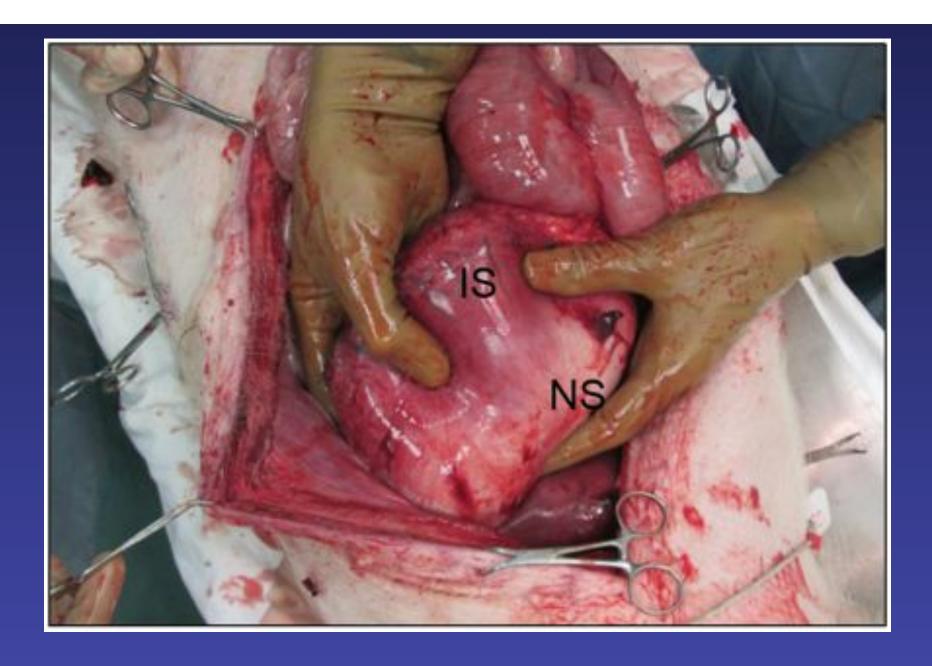
Cardiac (RV) embolus (red clot) At Necropsy
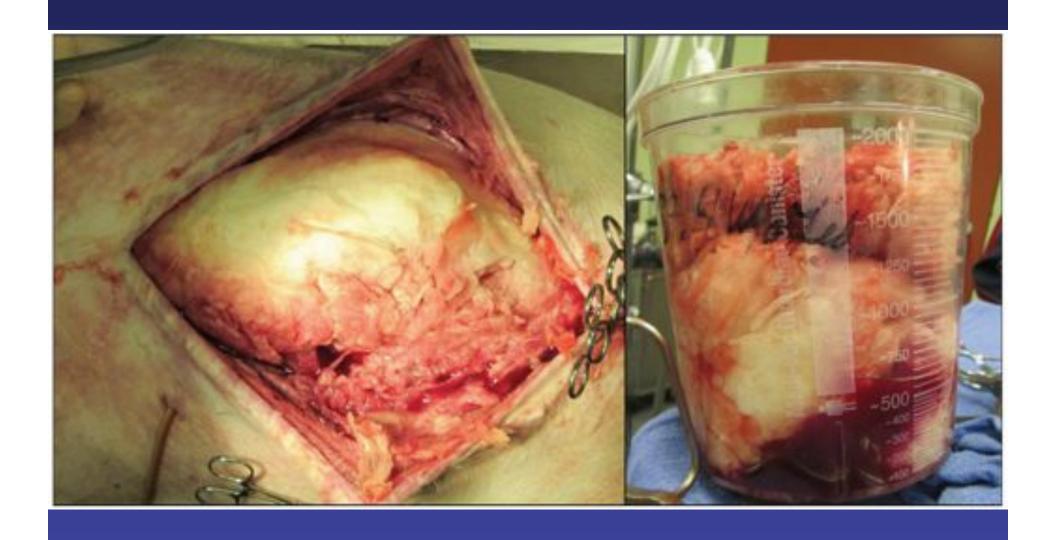
Cardiac (RF) Embolus: Foam
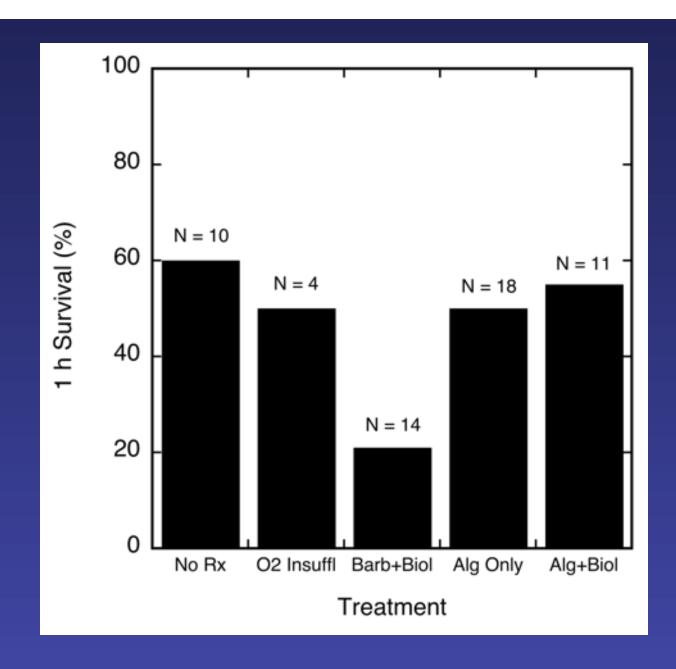
Intestinal Discoloration From Excess Free CaCl$_2$
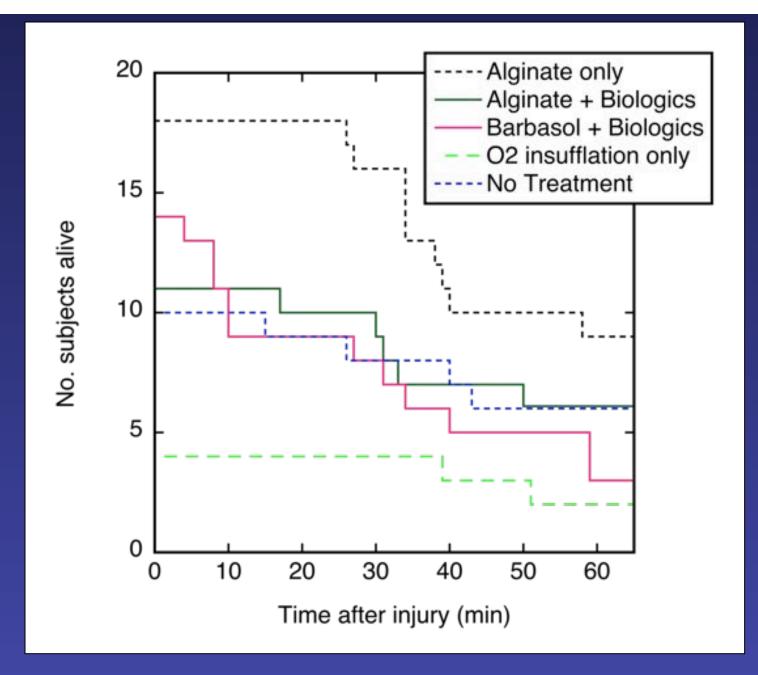
Stomach Discoloration From Excess Free CaCl$_2$
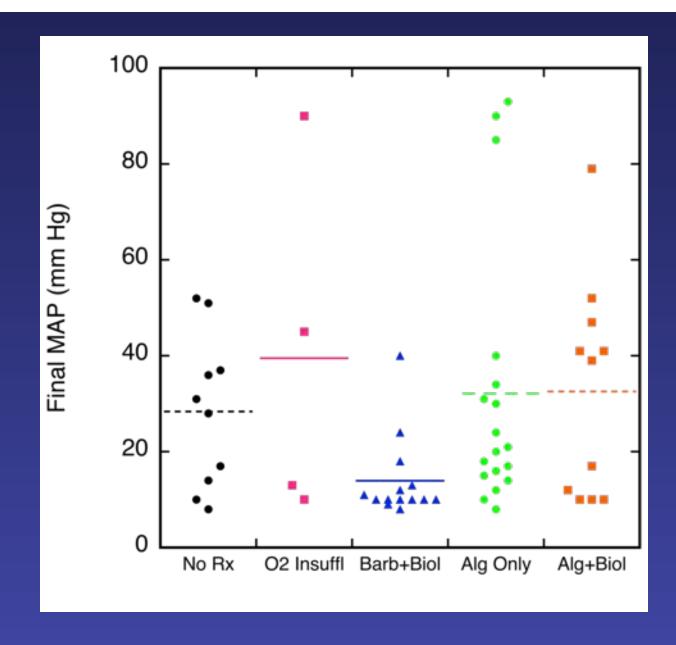
Evacuation Of Calcium Alginate Foam At Necropsy
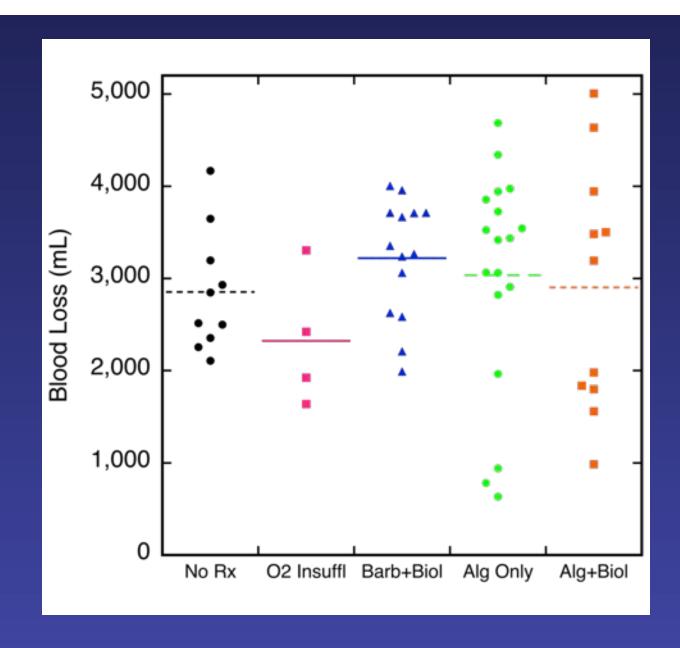
Survival at one hour post injury
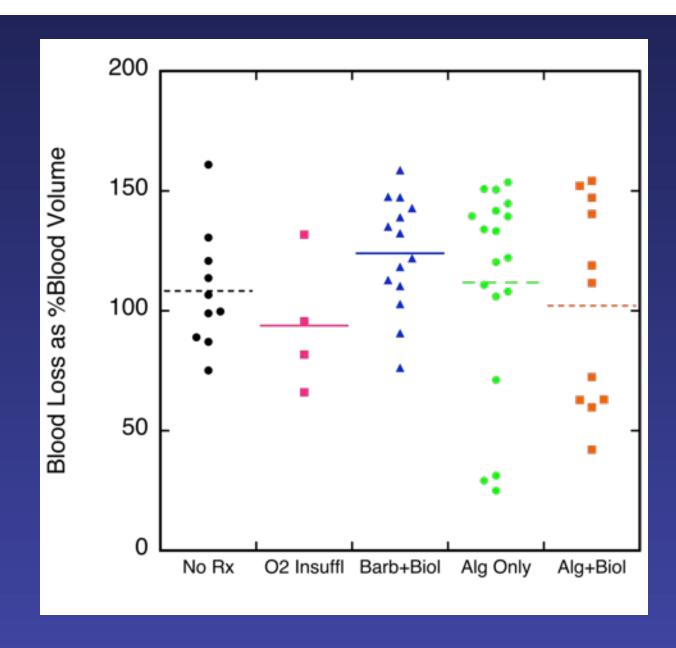
Kaplan Meier Survival Curve: Four Treatments
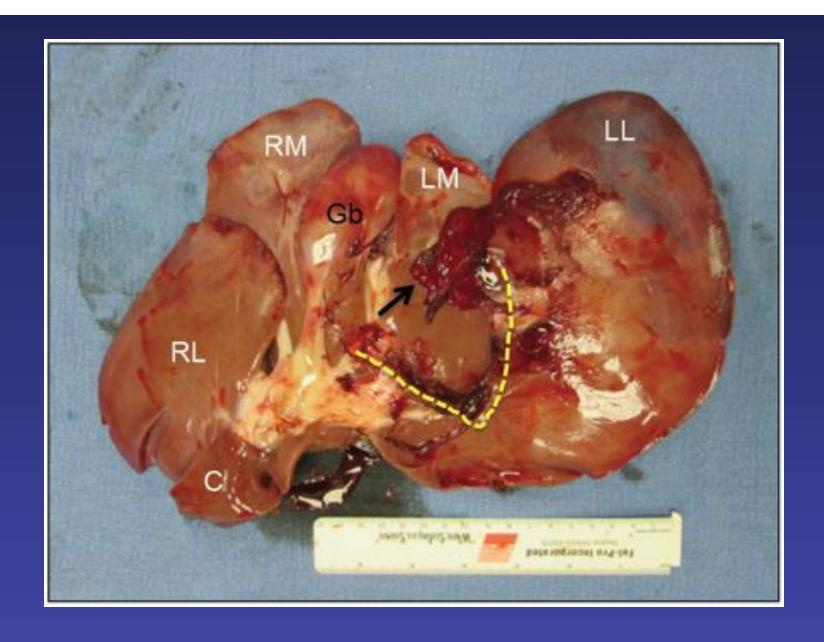
Final MAP at time of death

Blood Loss (absolute volume)

Blood Loss, expressed as % of blood volume

Liver *Ex Vivo*, LLLH Mechanism, 180 min Survivor

Standard Resuscitation Protocol

- Warm LR, given at 150 mL/min if MAP is <80% of pre-injury MAP
- Limit of 100 mL/kg
- For 40 kg subject, 4.0 L, completely given in <0.5 h.

"Hypotensive" Resuscitation Protocol

- Fluid rate = Animal wt (kg) x 100 mL/kg ÷ 180 min; administer when MAP is <80% of pre-injury MAP
- For a 40 kg subject, IVF rate ~22 mL/min
- Same total fluid volume

Part 3

Development of a Surgicel® mimic

Table 2. Cost of Surgicel® through various online vendors (and the Omaha VA Medical Center).

Source	Cost per box	No.	Size	sq in/unit	cm2/unit	total cm2	$/cm2
Save Rite Medical	$950.00	24	4x8in	32	206.4	4953.6	0.19
Just For Medical Supplies	$999.99	24	4x8in	32	206.4	4953.6	0.20
Omaha VAMC	$960.00	12	2x14in	28	180.6	2167.2	0.44
HomeHealthMedical.com	$2,906.99	24	4x8in	32	206.4	4953.6	0.59
Omaha VAMC	$1,272.00	12	4x8in	28	180.6	2167.2	0.59
Just For Medical Supplies	$1,103.99	12	3x4in	12	77.4	928.8	1.19
4MD Medical Solutions	$188.08	24	0.5x2in	1	6.5	154.8	1.21
4MD Medical Solutions	$2,277.26	24	3x4in	12	77.4	1857.6	1.23
Save Rite Medical	$653.90	12	2x3in	6	38.7	464.4	1.41
Just For Medical Supplies	$653.90	12	2x3in	6	38.7	464.4	1.41
Healthcare Supply Pros	$1,783.28	24	2x3in	6	38.7	928.8	1.92
DealMed Medical Supplies	$344.10	12	0.5x2in	1	6.5	77.4	4.45
4MD Medical Solutions	$692.15	24	0.5x2in	1	6.5	154.8	4.47
Healthcare Supply Pros	$419.97	12	0.5x2in	1	6.5	77.4	5.43

Issue: relative high cost of Surgicel® ($200M Annual U.S. Market)
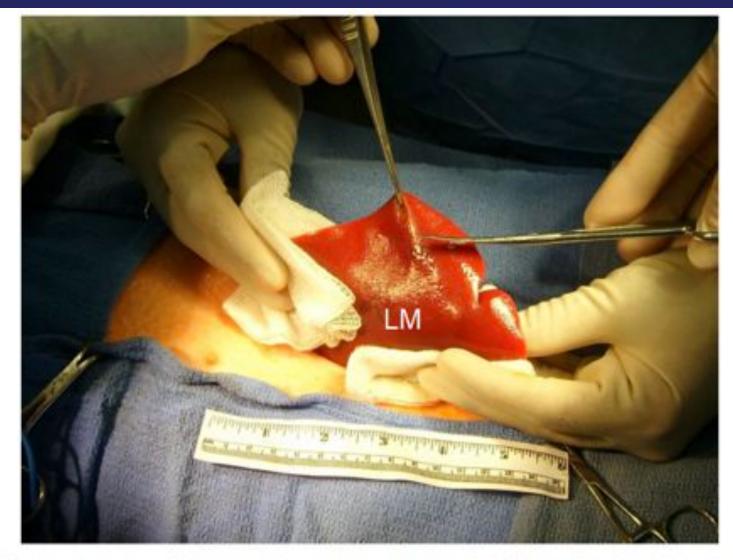
Figure 2. Excision technique. Scissors will cut a 6 cm strip from the LM lobe. Cephalad is to the left.

Figure 4. Post-treatment. Wound imaged face on, covered with Surgicel (S; turns blackish when contacted by blood). Cephalad is to the left. Packet of Surgicel® is shown in right panel.

Figure 2, Swine 132. Liver ex vivo, lateral view showing resection site of tip of left lateral (LL) lobe, covered with Surgicel (S).

Fig. 1. PCL mesh (test bandage for NEDED project). Single ply, ~20 x 10 cm (8 x 4 in). Inset: folded into thirds, ~10 x 6.7 cm (4 x 2.7 in), as used *in vivo*. Bandage wt ~0.5 g.
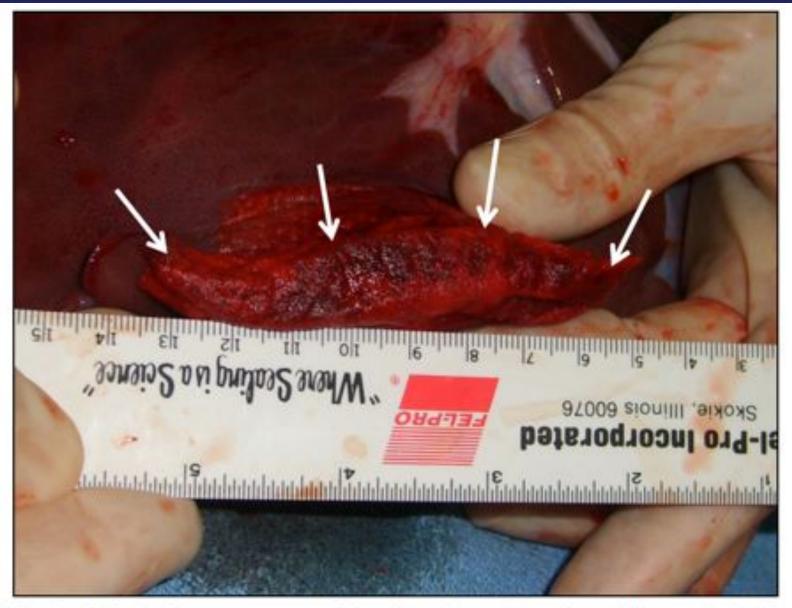
Fig. E2, Swine 149. *Ex vivo* appearance of dressing after euthanasia and liver explantation. Injury (arrows) on left lateral lobe viewed face-on with PCL gauze in place; cotton gauze in Fig. E1 has been removed.
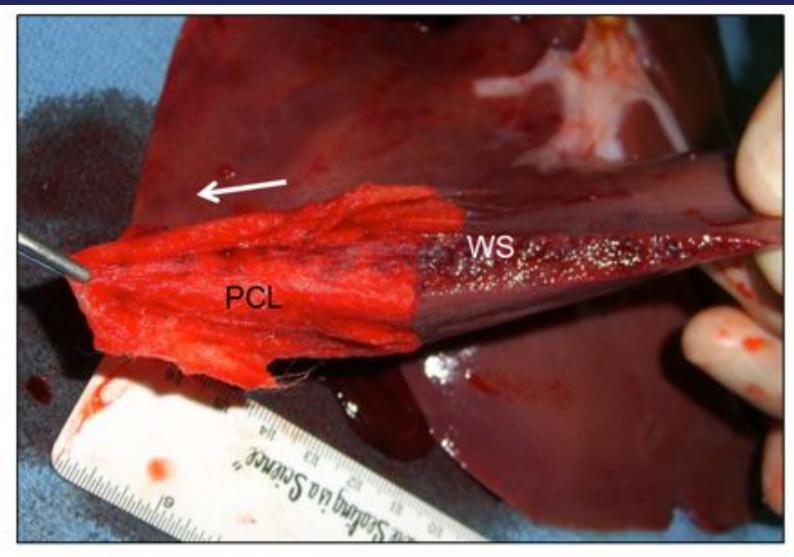
Fig. E3, Swine 149. Same view as Fig. E2; PCL gauze is being peeled away from wound surface (WS) in direction of arrow.
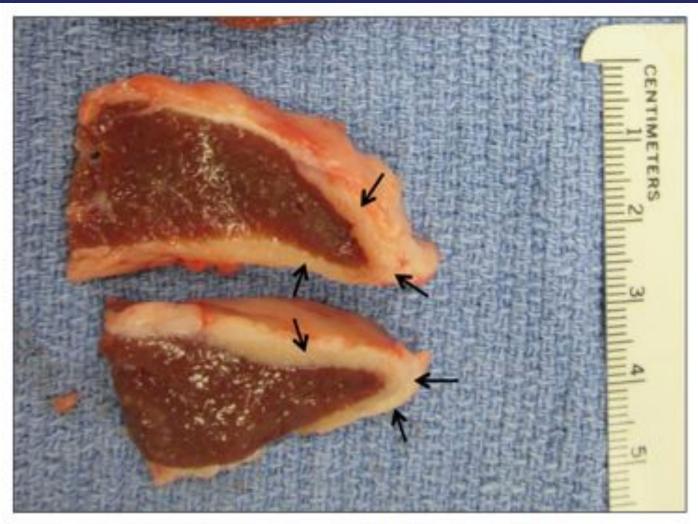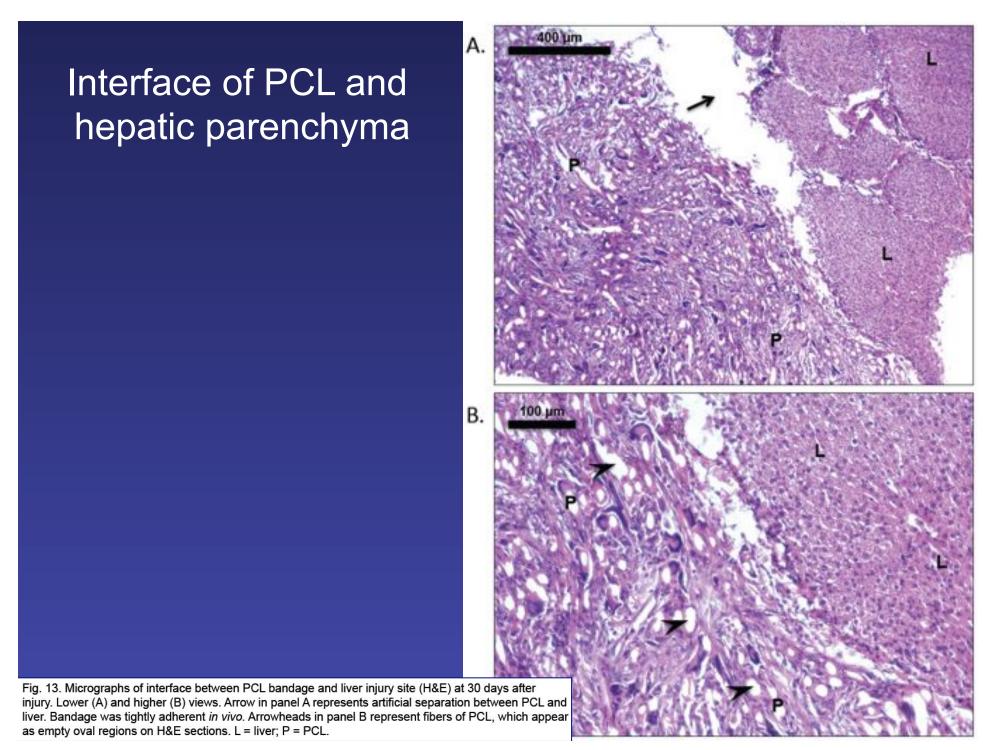
Fig. 11, Swine 184 necropsy. Close-up of two sections from Fig. 10, through liver with adherent PCL (arrows).

Interface of PCL and hepatic parenchyma

Fig. 13. Micrographs of interface between PCL bandage and liver injury site (H&E) at 30 days after injury. Lower (A) and higher (B) views. Arrow in panel A represents artificial separation between PCL and liver. Bandage was tightly adherent *in vivo*. Arrowheads in panel B represent fibers of PCL, which appear as empty oval regions on H&E sections. L = liver; P = PCL.

Title: Comparison of a Synthetic Resorbable Bandage vs. Oxidized Regenerated Cellulose for Treatment of Minor Hemorrhage in a Porcine Model

Author list: Ujwal R. Yanala, Sandra Noriega, Ruben Spretz, Jorge Ragusa, Luis Nuñez, Gustavo Larsen, Mark A. Carlson

Affiliations:
MAC, URY: UNMC & VAMC
SN, RS, JR, LN: LNK Chemsolutions
GL: LNK Chemsolutions and Dept Chemical & Biomolecular Engineering, UNL

Background: Our objective was to compare the efficacy and toxicity of a synthetic resorbable hemostatic bandage vs. an analogous commercial product in a porcine model of minor hepatic resection.
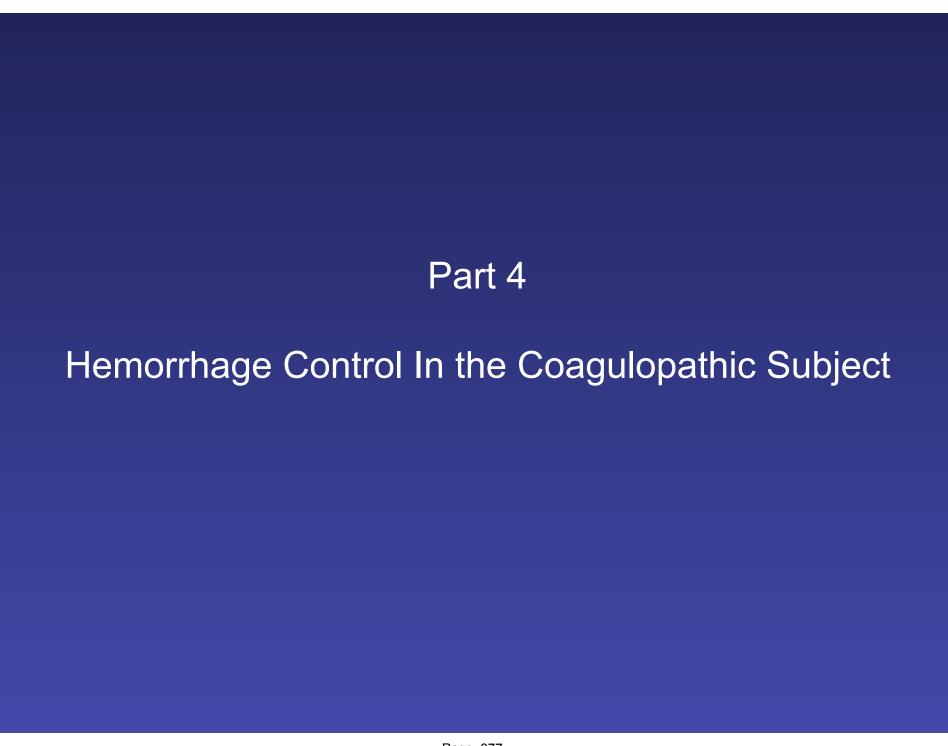
Methods: For the nonsurvival efficacy study, anesthetized boars (3 months, 29-40kg) underwent arterial/venous line placement and splenectomy. A 1x8cm section of liver was resected from the edge of the left lateral lobe, and test bandage (macroporous polycaprolactone mesh, PCL; N=10) or oxidized regenerated cellulose (ORC; Surgicel®, Ethicon®; N=10) was applied with manual pressure for 5 minutes. Resuscitation then was performed with warm LR (target=80% preinjury MAP), and blood loss was measured 60 min after injury. For the survival toxicity study, a similar resection technique was employed (N=6 for each material), and necropsy was performed at 30 days to evaluate for bandage toxicity (subject growth, serum chemistry, histology).
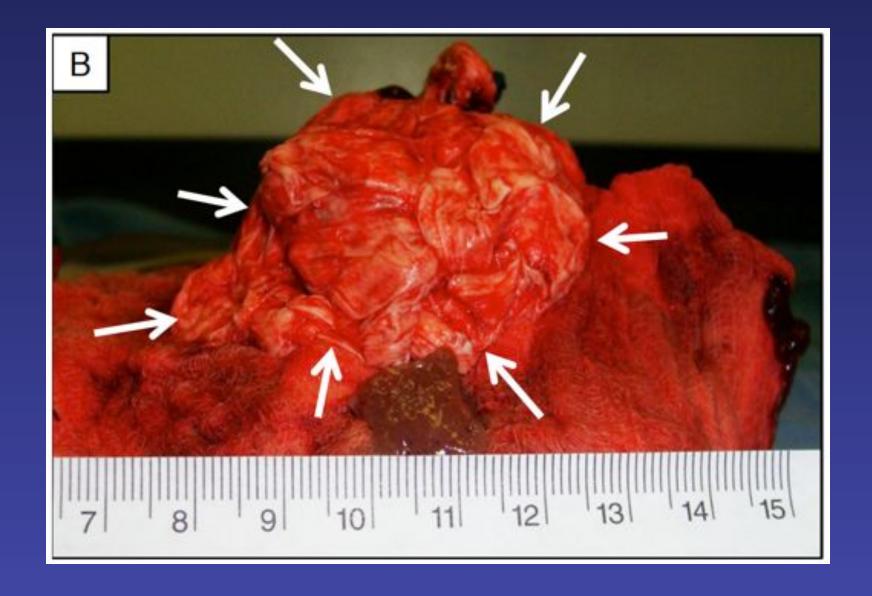
Results: Pre-injury weight, VS, and laboratory testing did not differ among groups. Resection mortality was zero. In the efficacy study, there were no differences between the PCL vs. ORC groups in blood loss or other post-injury variables (Table), except that the resuscitation fluid volume in the ORC group was greater. Other than minor granuloma formation at the implantation site with both PCL and ORC, the survival study did not reveal any measurable toxicity.

Conclusion: The efficacy and toxicity of the PCL test bandage vs. the ORC comparator were not different. The PCL bandage could represent a lower-cost alternative to ORC for minor surgical bleeding.

Table. Data (mean±sd) from efficacy study at the 60 min end-point.

Variable	PCL	ORC	unpaired t-test
Resection mass (g)	7.6 ± 1.9	6.7 ± 2.0	0.32
Blood Loss (mL)	93 ± 27	111 ± 55	0.38
Resuscitation (mL)	594 ± 425	1952 ± 1363	0.01*
MAP (mm Hg)	89 ± 8	93 ± 11	0.30
Base excess (mEq/L)	4.1 ± 0.8	4.5 ± 1.6	0.54
Hb (g/dL)	12.8 ± 1.2	12.9 ± 0.9	0.85
Fibrinogen (mg/dL)	88 ± 15	88 ± 22	0.97
Platelets (1,000/µL)	266 ± 56	327 ± 94	0.11
INR	1.0 ± 0.1	1.0 ± 0.0	0.14

Part 4

Hemorrhage Control In the Coagulopathic Subject

Plan:

Study Bandage-Wound Adhesion Phenomena

Method:

Ex Vivo Adhesion Testing
(tissue strips & bandage materials)

Tensiometer:
DURIP & ShEEP mechanism

"That's all folks!"

People

Lab
Jie Chao
Tiffany Peña
Dean Heimann
Chris Hansen
Ujwal Yanala
Crystal Cordes
Amy Prall
Mark Eichler
Jeremiah Gums
Mohammed Foda
Alex Lesiak
David Doyle

UNMC
Iraklis Pipinos
Jason Johanning
Jon Thompson
Jim Eudy
Lynette Smith

Lincoln
Bill Velander
Gustavo Larsen
Jennifer Calcaterra
Sandra Noriega
Ruben Spretz
Luis Nuñez
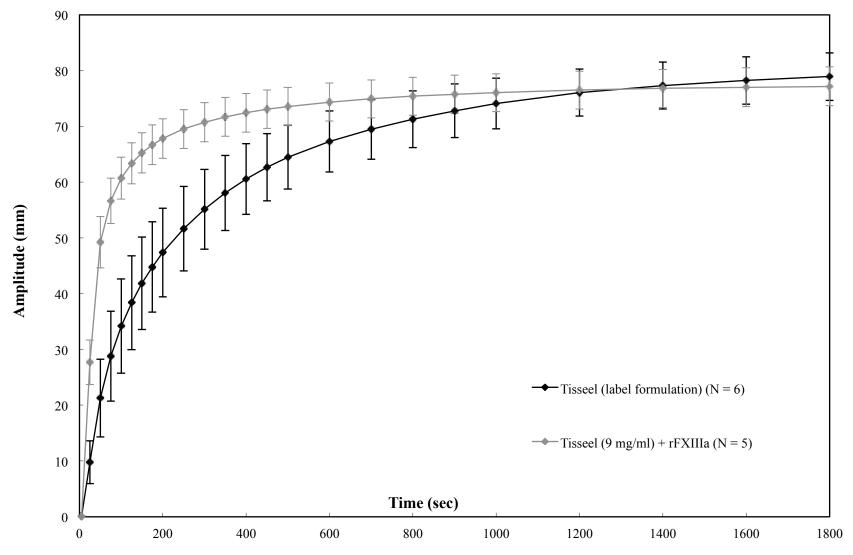
Dual fibrin-sealant-alginate-foam (DFSF) applicator

Fibrin Sealant for Gauze and Foam Applications

- Dual fibrin-sealant-alginate-foam (DFSF) uses pd-F1, r-FIIa, r-FXIIIa
- pd-LFS contains FN
- pd-F1:pd-Fibronectin complex
- Switch to r-LFS in DFSF after pd-LFS

Viscosity effects: rFXIIIa relieves diffusion slowed crosslinking reactions during fibrin formation
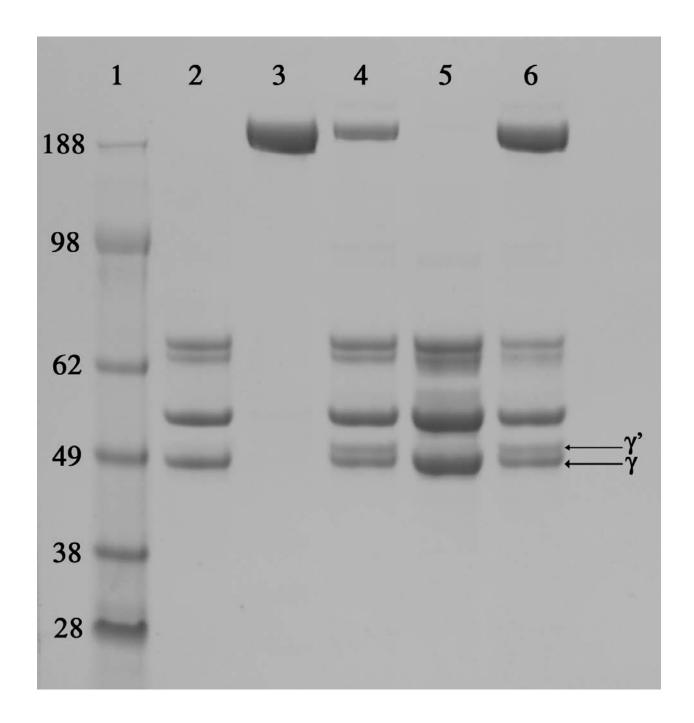
rFXIIIa can decrease the amount of pdF1 needed while improving kinetics
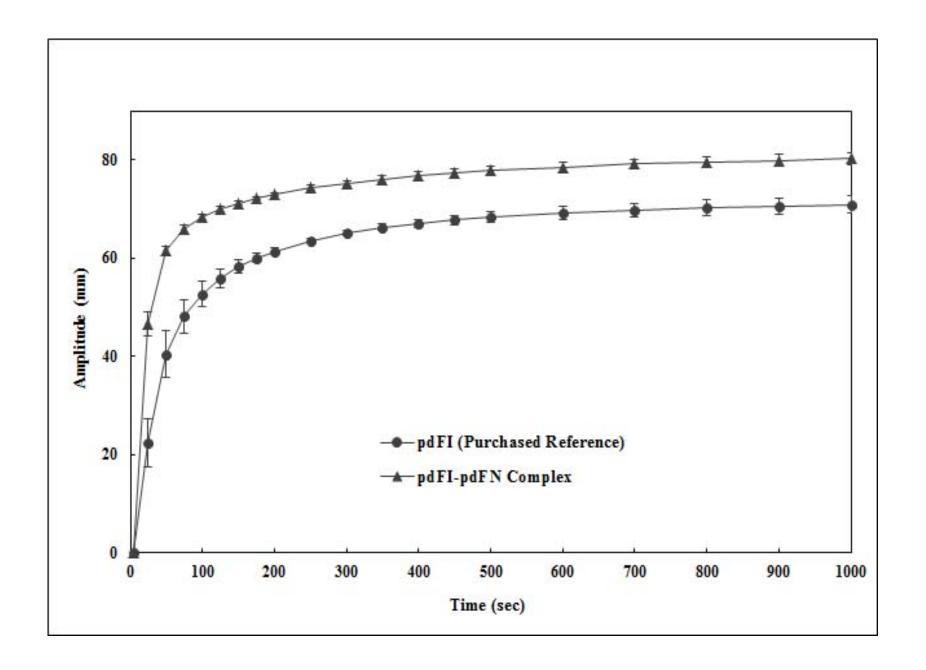
DFSF experiments
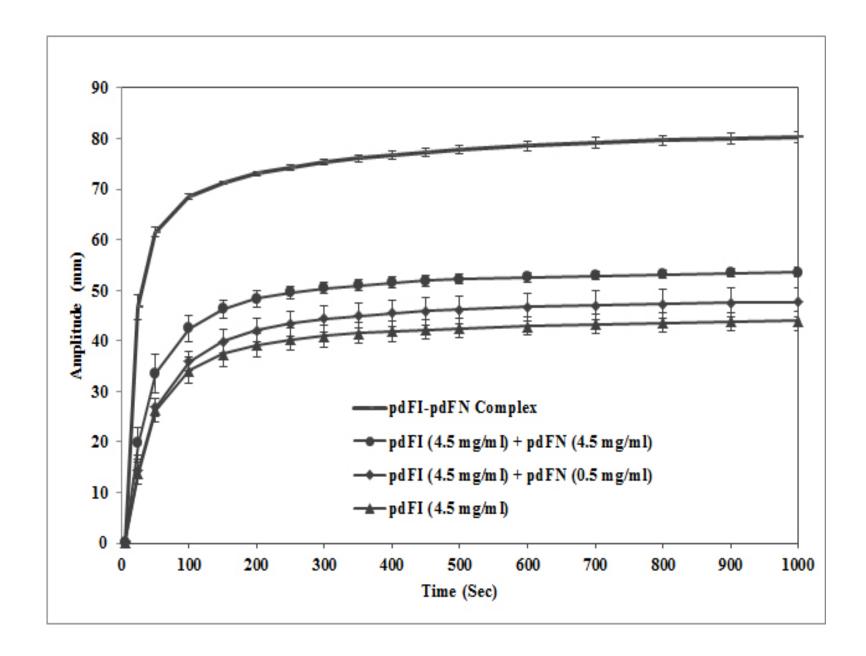
- Use pd-LFS for early development
  - Made from USARMY plasma
  - Cost effective @ >100 g quantities
- Switch to r-LFS once application is matured
  - Incompressible wound model
  - Application volume of Foam and LFS
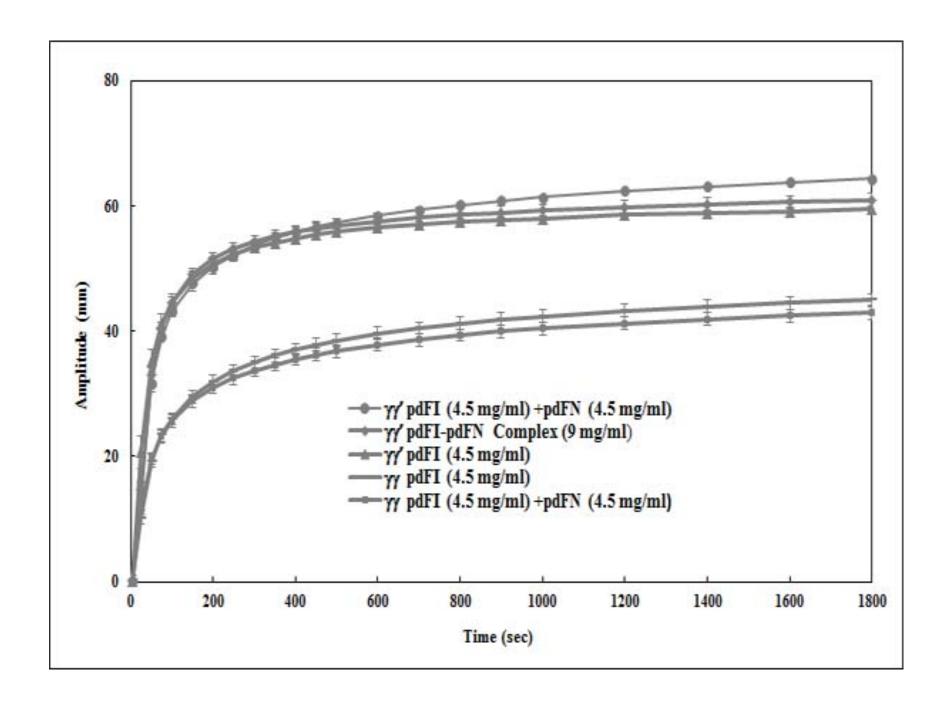
Fibronectin content of pd-LFS

- Basic discovery of pd-F1:FN complex

- All ΥΥ'-heterodimer pd-F1 is complexed with FN

- FN doesn't adversely affect clot strength

- ΥΥ'-heterodimer pd-F1 is main source of TEG strength

- ΥΥ'-heterodimer pd-F1 : F1 tight complex

- ΥΥ-heterodimer pd-F1 :FN weak interaction

- ΥΥ'-heterodimer r-F1: FN forms complex

Legend (within figure):
- γγ′ pdFI (4.5 mg/ml) +pdFN (4.5 mg/ml)
- γγ′ pdFI-pdFN Complex (9 mg/ml)
- γγ′ pdFI (4.5 mg/ml)
- γγ pdFI (4.5 mg/ml)
- γγ pdFI (4.5 mg/ml) +pdFN (4.5 mg/ml)

Axes: X-axis: Time (sec); Y-axis: Amplitude (mm)
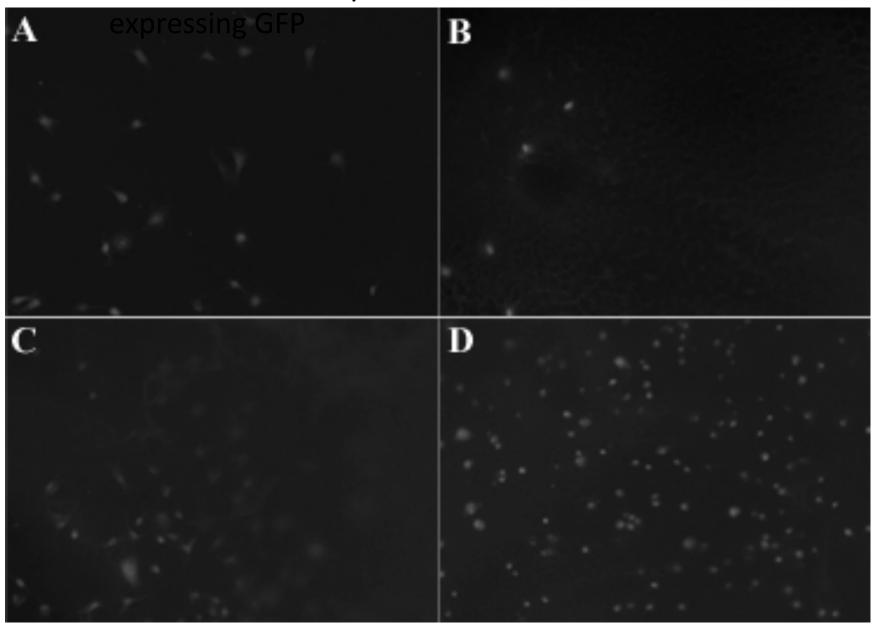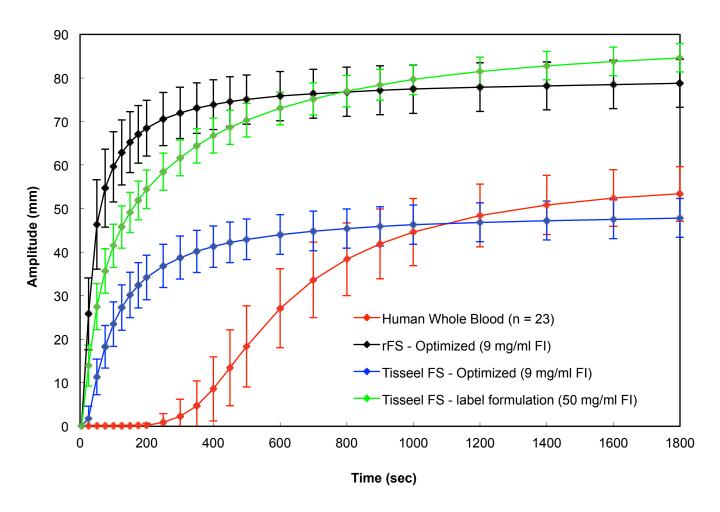
Cell Adhesion assay for GFP transfected fibroblasts expressing GFP
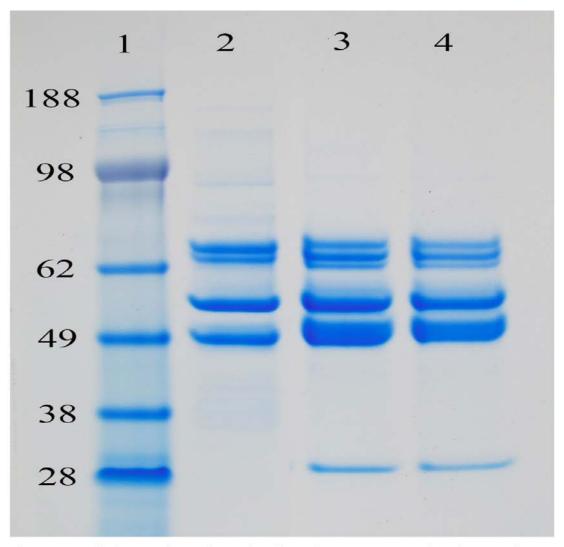
Figure 1: Gel electrophoresis evaluation of rFI. Lane 1: molecular weight marker; lane 2: pdFI (ER); lane 3: rFI (least acidic fraction); lane 4: rFI (most acidic fraction).
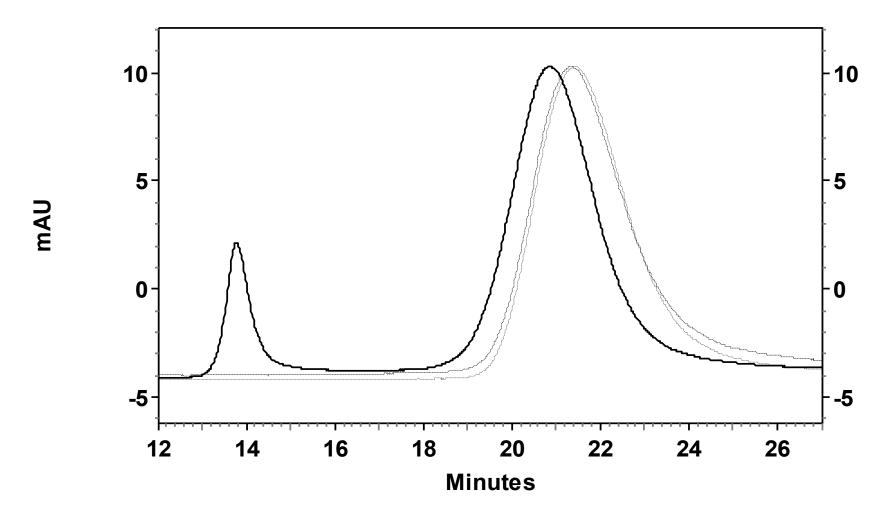
Figure 2: SEC analysis of ER pdFI (solid), most acidic rFI (dot), and least acidic rFI (dash)
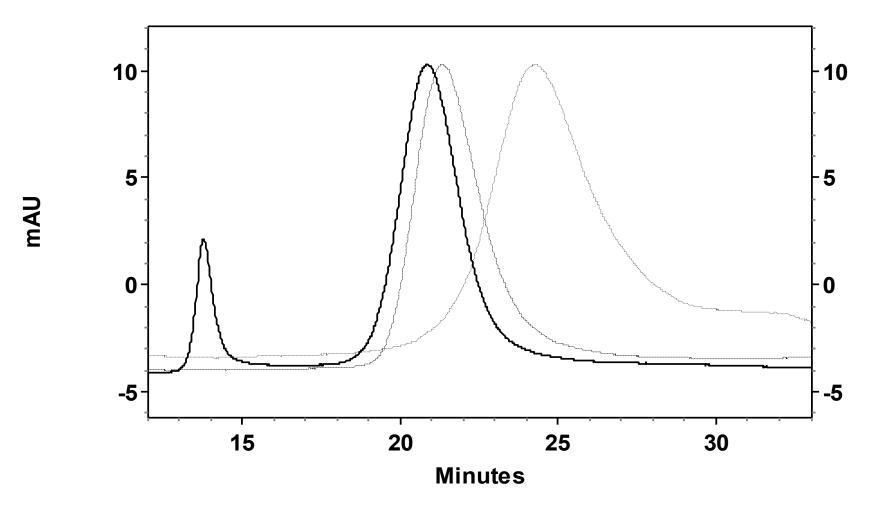
Figure 3: SEC analysis of ER pdFI (solid), most acidic rFI (dash), and pdFN (dot).
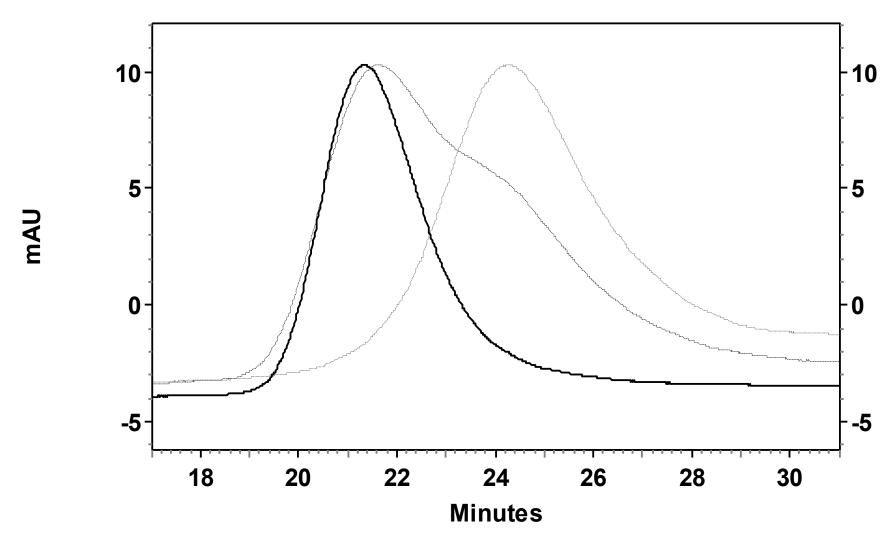
Figure 4: SEC analysis of most acidic rFI (solid), most acidic rFI + pdFN (dash), and pdFN (dot).
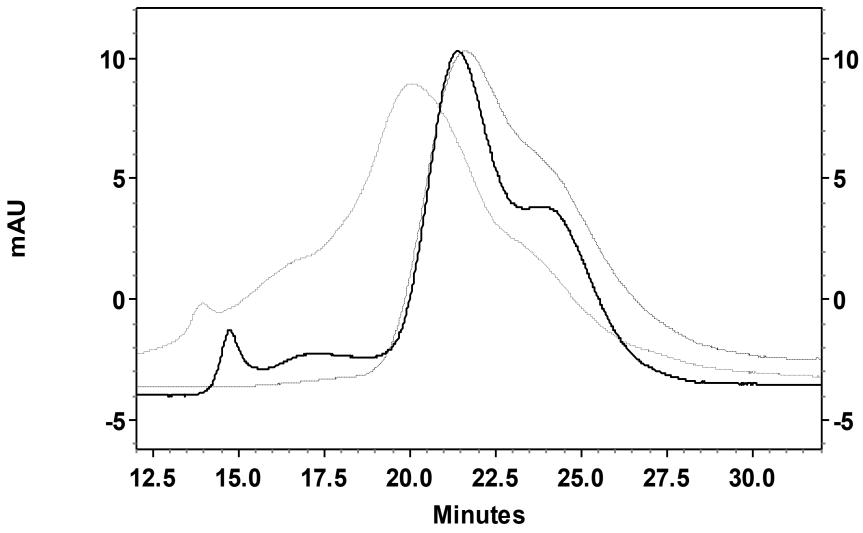
Figure 5: SEC analysis of native pdFI-pdFN complex (solid), γγpdFI + pdFN (dot), and most acidic rFI + pdFN (dash).

Figure 6: SEC analysis of native pdFI-pdFN complex (solid), $\gamma\gamma'$pdFI + pdFN (dot), and most acidic rFI + pdFN (dash).

Fibronectin content of pd-LFS

- Basic discovery of pd-F1:FN complex
- All ϒϒ'-heterodimer pd-F1 is complexed with FN
- FN doesn't adversely affect clot strength
- ϒϒ'-heterodimer pd-F1 is main source of TEG strength
- ϒϒ'-heterodimer pd-F1 : F1 tight complex
- ϒϒ-heterodimer pd-F1 :FN weak interaction
- ϒϒ'-heterodimer r-F1: FN forms complex

Future Work and Discussions
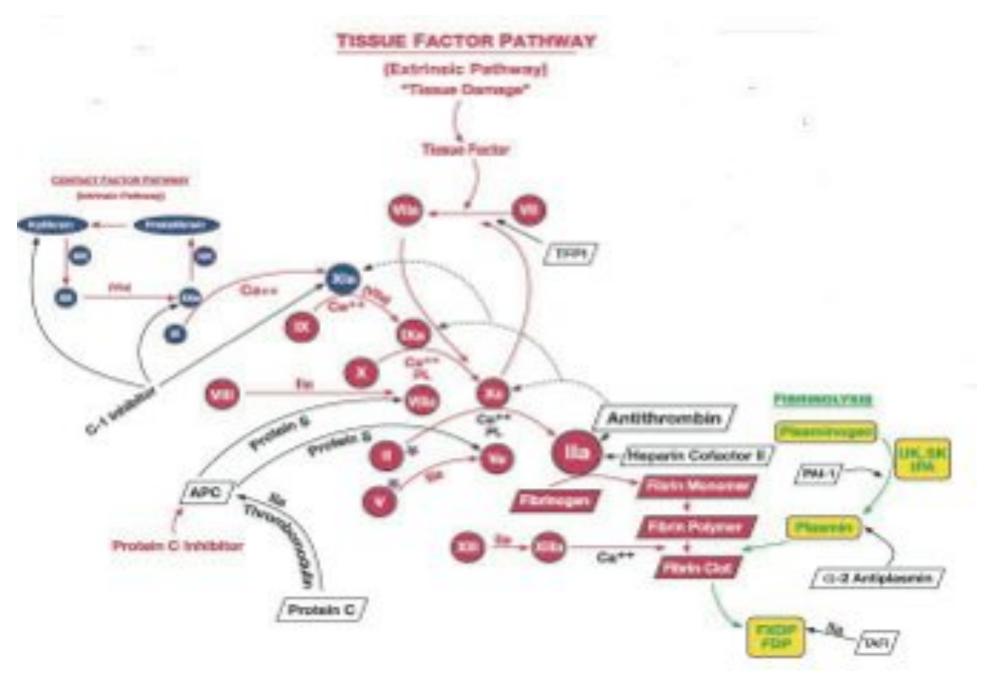
- pd-F1: FN complex presentation at the International Fibrinogen Workshop in Marseille France July 8-10

- Brazilian Ministry of Health:UNL:USARMY collaboration (ARO, ISR, Other)

Treatment of Hepatic Resection in Swine using Novel Delivery Methods for Fibrin Sealant

Porcine Surgical Trauma Models
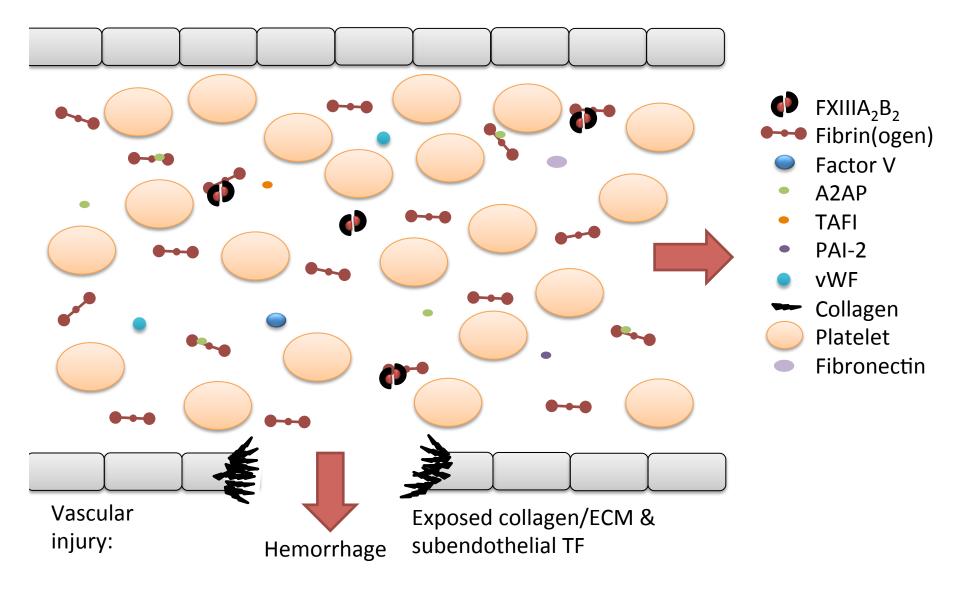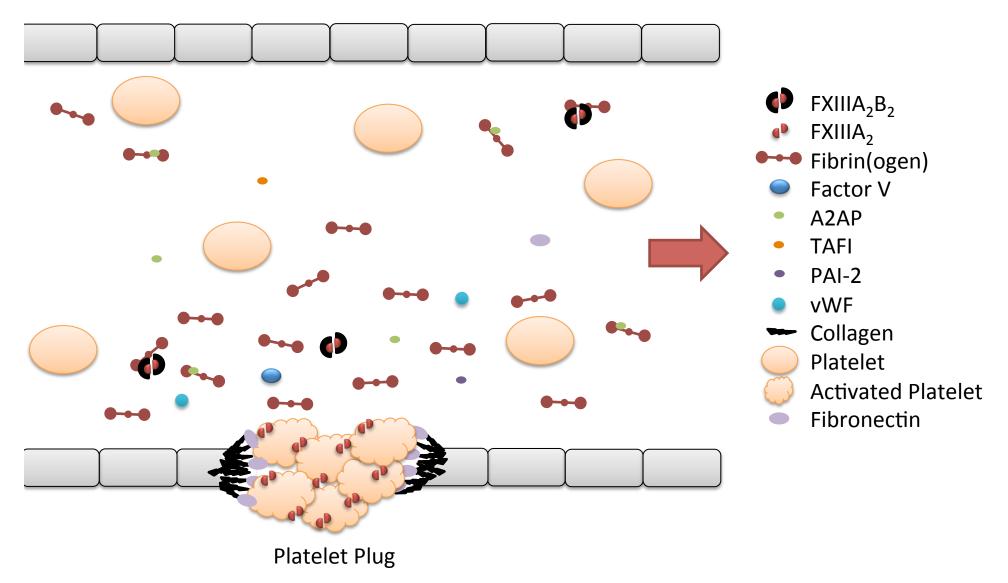
Hemostasis is complex and many its proteins have dual roles in healing

Vascular Injury & Platelet activation

Legend:
- FXIIIA$_2$B$_2$
- Fibrin(ogen)
- Factor V
- A2AP
- TAFI
- PAI-2
- vWF
- Collagen
- Platelet
- Fibronectin

Vascular injury:

Hemorrhage

Exposed collagen/ECM & subendothelial TF

Primary Hemostasis

Platelet Plug

	$FXIIIA_2B_2$
	$FXIIIA_2$
	Fibrin(ogen)
	Factor V
	A2AP
	TAFI
	PAI-2
	vWF
	Collagen
	Platelet
	Activated Platelet
	Fibronectin
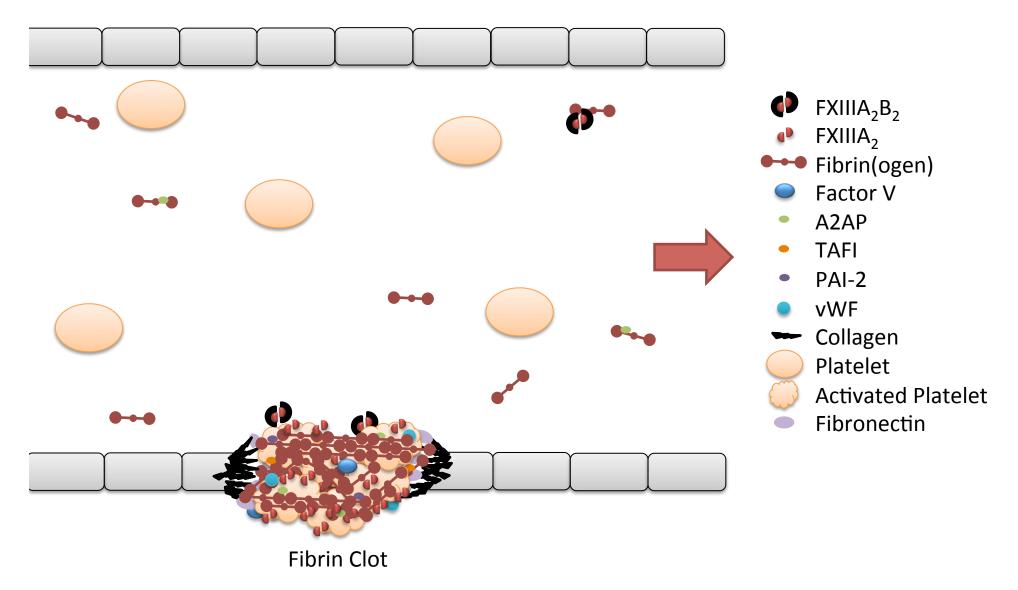
Secondary Hemostasis

Fibrin Clot

Legend:
- FXIIIA$_2$B$_2$
- FXIIIA$_2$
- Fibrin(ogen)
- Factor V
- A2AP
- TAFI
- PAI-2
- vWF
- Collagen
- Platelet
- Activated Platelet
- Fibronectin
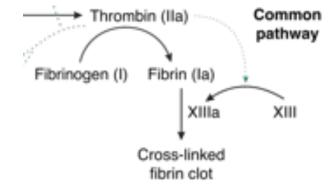
Fibrin Sealant

Fibrinogen (Plasma-derived or Recombinant)
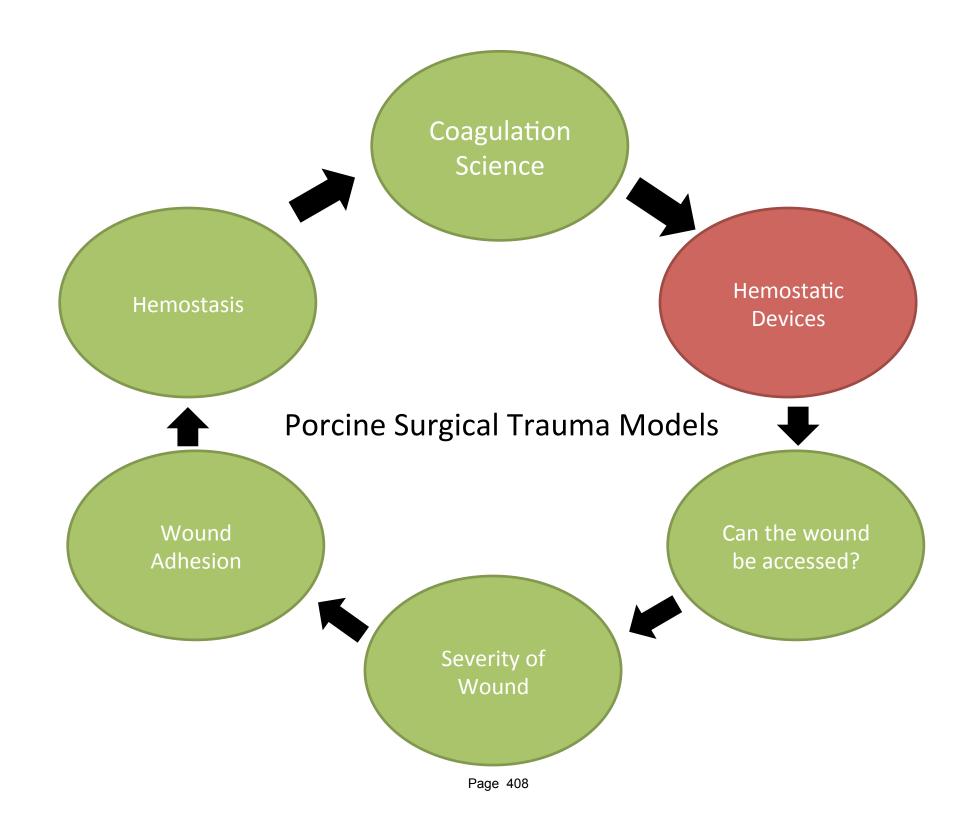
Thrombin (Commercial Recombinant)

Factor XIII (Recombinant)
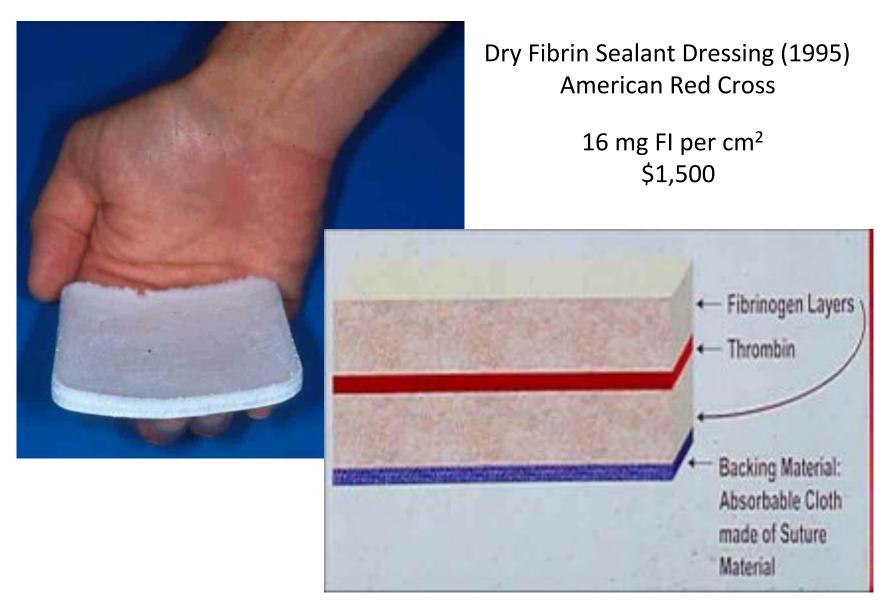
Fibrinogen Source:
SCNT Cloned Swiss Brown Dairy cows

Porcine Surgical Trauma Models
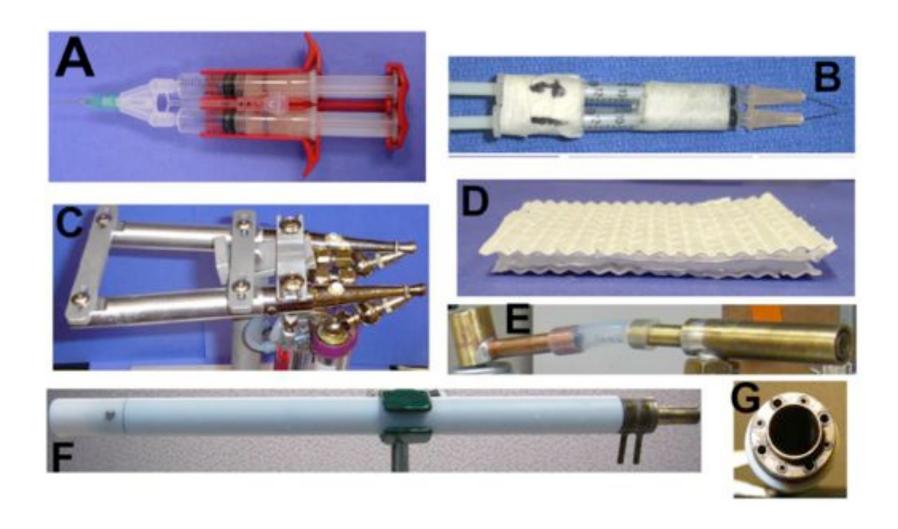
ARC Dry Fibrin Sealant Dressing(DFSD)

Dry Fibrin Sealant Dressing (1995)
American Red Cross

16 mg FI per cm²
$1,500

Fibrinogen Layers

Thrombin

Backing Material:
Absorbable Cloth
made of Suture
Material

Field Use of ARC Dry FS Bandage

- August 2003
  - US Army Special Forces Unit ambushed in Tikrit, Iraq
  - 29 year old male: multiple gunshot wounds to both thighs and left shoulder
- Control
  - Right thigh + shoulder: adequate haemostasis with standard field dressings
  - Unable to control femoral arterial bleeding from left thigh wounds with standard pressure dressing and tourniquet
- Experimental: ARC dressing applied to left thigh resulting in haemostasis
- 12 months later: Subject back in the field
- **Army deemed not feasible due to cost ($1,500/unit)**
- **Need a more pliable device for tortuous wounds**

Fibrin Sealant Delivery Devices

Spray Device for Application of FS on Bandages
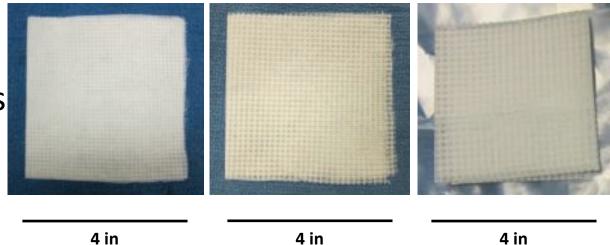
Bioresorbable Bandages

- Nanofiberous PLA

- PCL
  - Small pores
  - Midsize pores
  - Large pores
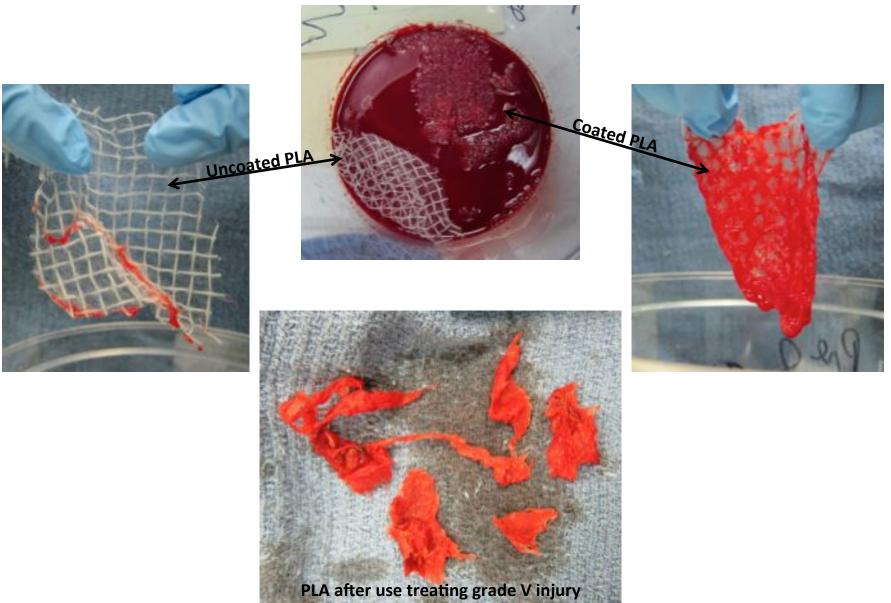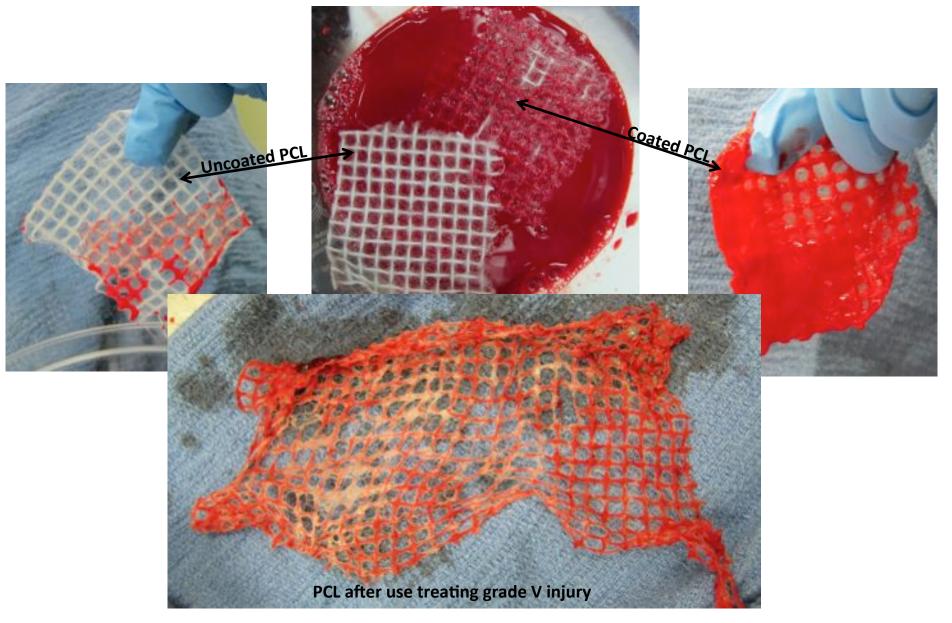
	4 in	4 in	4 in
Pores:	0.2 mm x 0.2 mm	0.3 mm x 0.3 mm	0.4 mm x 0.4 mm

PLA hydrophobic vs hydrophilic

Uncoated PLA

Coated PLA

PLA after use treating grade V injury

PCL hydrophobic vs hydrophilic

Uncoated PCL

Coated PCL

PCL after use treating grade V injury
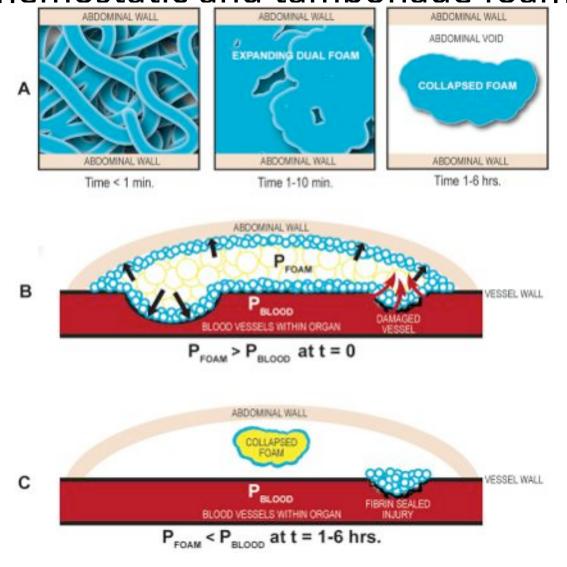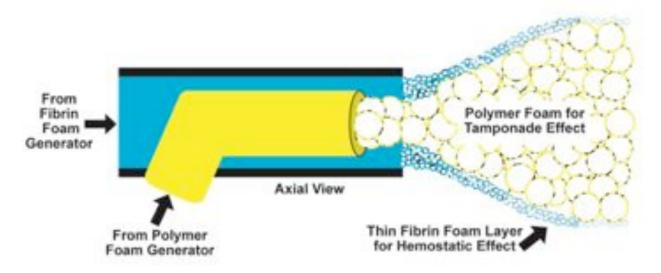
Engineered hydrodynamics/hydrostatics of co-administered reabsorbable polymer particulate hemostatic and tamponade foams
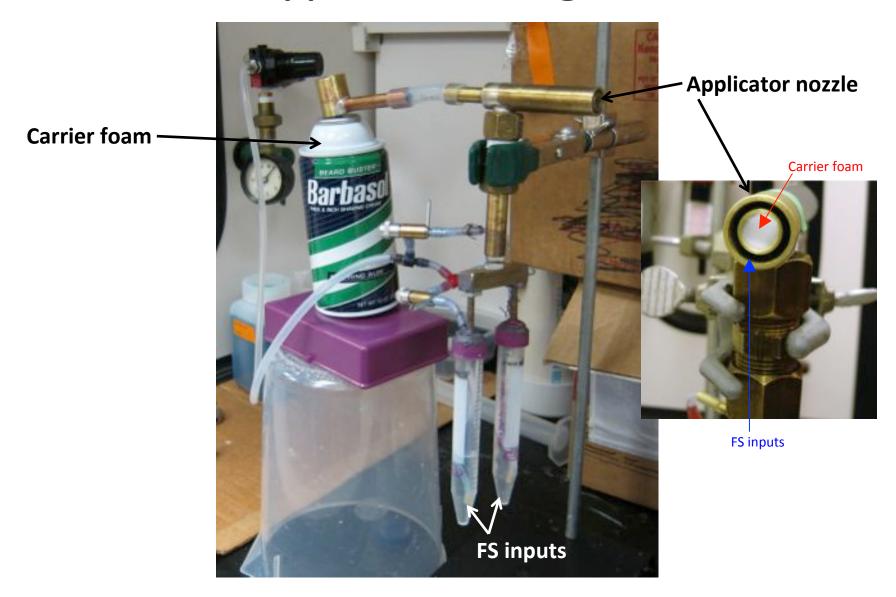
Prototype Foaming Device

Concentric Annular Polymer Particulate Foam Dispenser

From Fibrin Foam Generator

From Polymer Foam Generator

Axial View

Polymer Foam for Tamponade Effect

Thin Fibrin Foam Layer for Hemostatic Effect

Concentric Hemostatic and Tamponade Polymer Particulate Foam Jets

Radial View of Foam Dispenser

Prototype Foaming Device

Carrier foam

Applicator nozzle

Carrier foam

FS inputs

FS inputs

Prototype Device: Carrier Foam
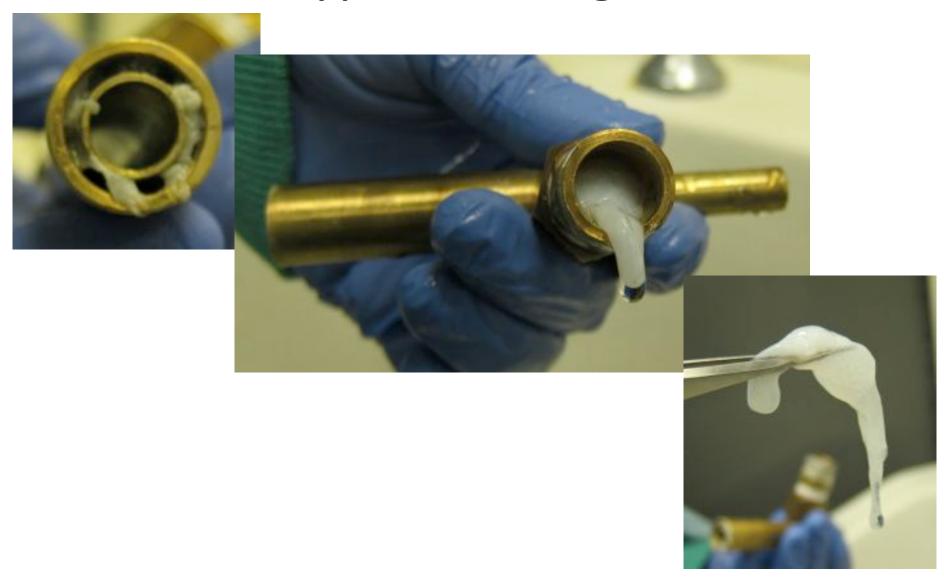
1 Input (Blue)
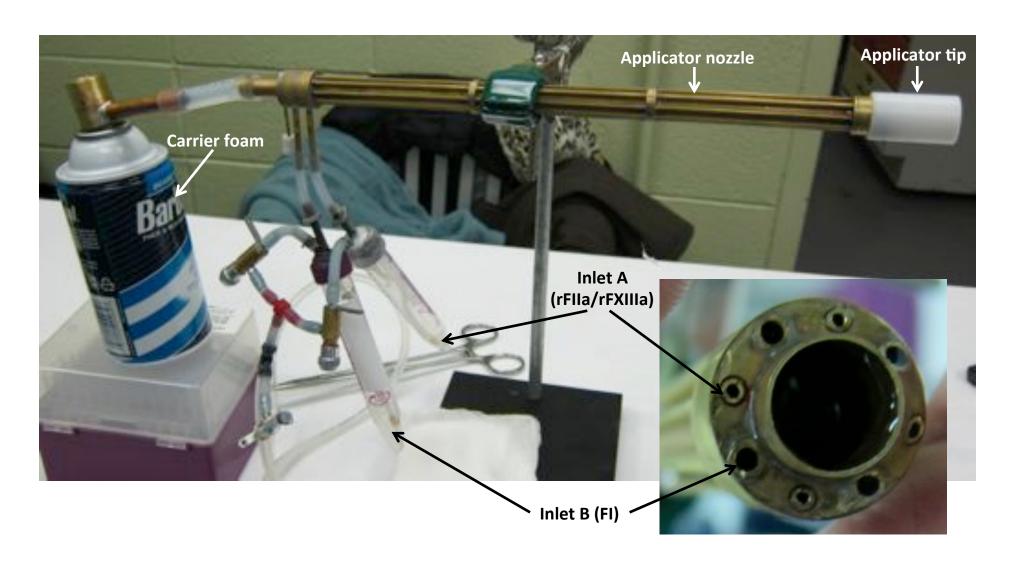
**2 Inputs
(Blue + Red)**
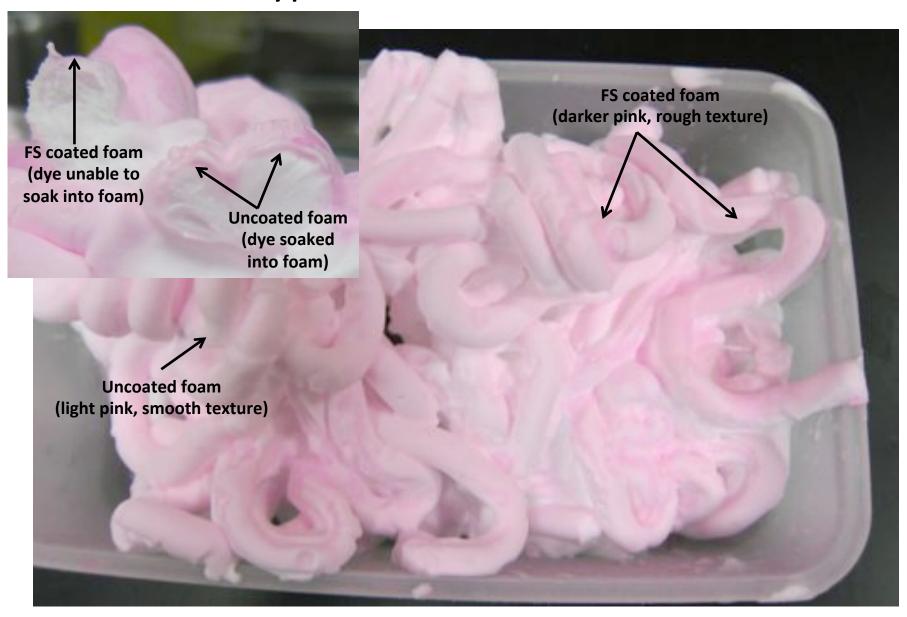
Prototype Foaming Device
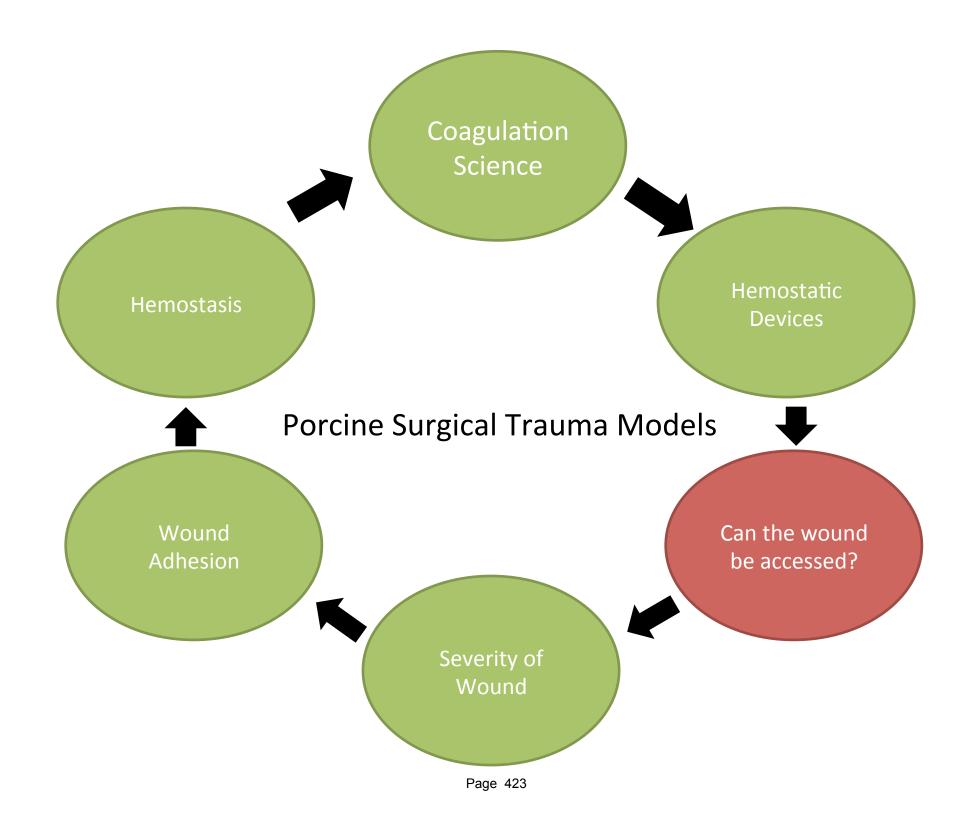
2nd Generation Foaming Device

Carrier foam

Applicator nozzle

Applicator tip

Inlet A (rFIIa/rFXIIIa)

Inlet B (FI)

Prototype Device: Carrier Foam + FS

FS coated foam (dye unable to soak into foam)

Uncoated foam (dye soaked into foam)

Uncoated foam (light pink, smooth texture)

FS coated foam (darker pink, rough texture)

Porcine Surgical Trauma Models

Treatment Process for Accessible Wounds

Spray FI or FI/FN on bandage
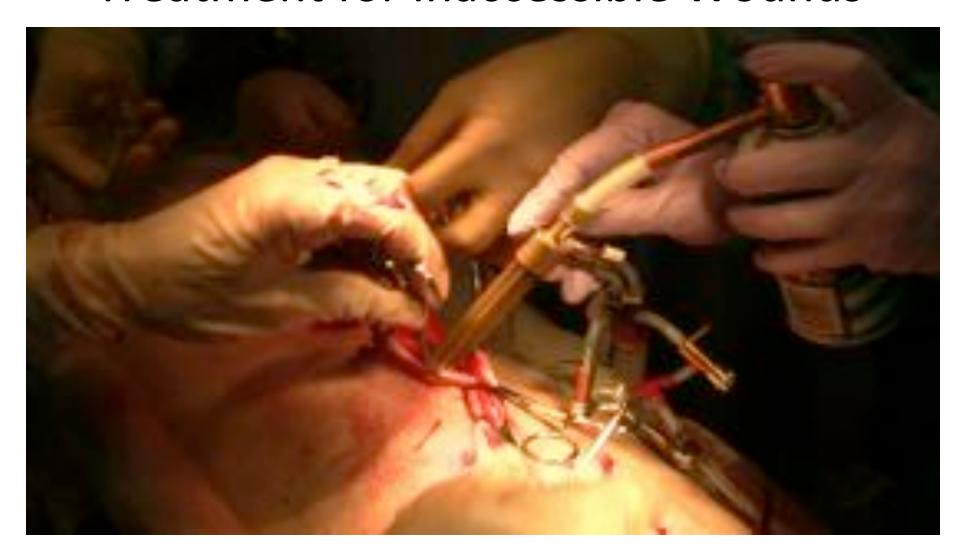
Make injury and spray rFXIIIa/rFIIa on bandage simultaneously

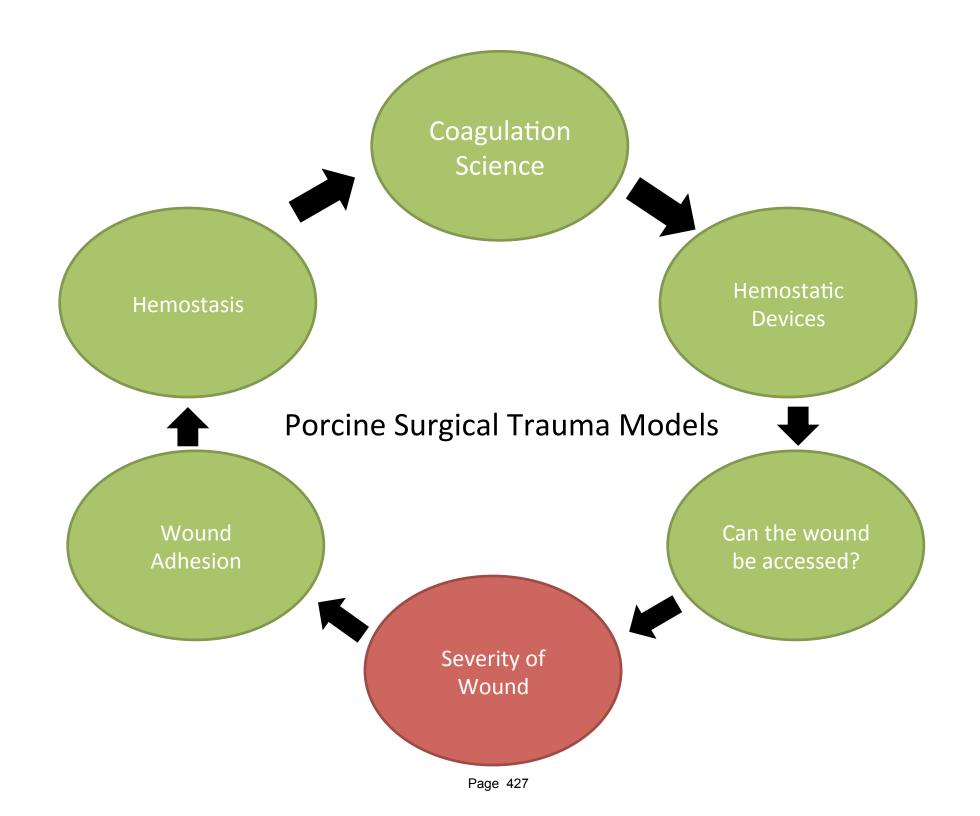Apply bandage with FS coated side toward wound

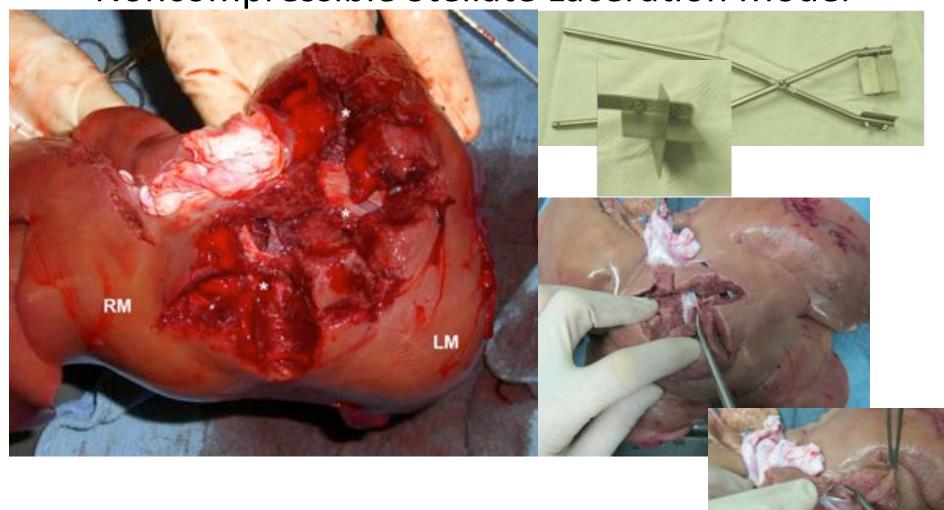Direct pressure for 5 min

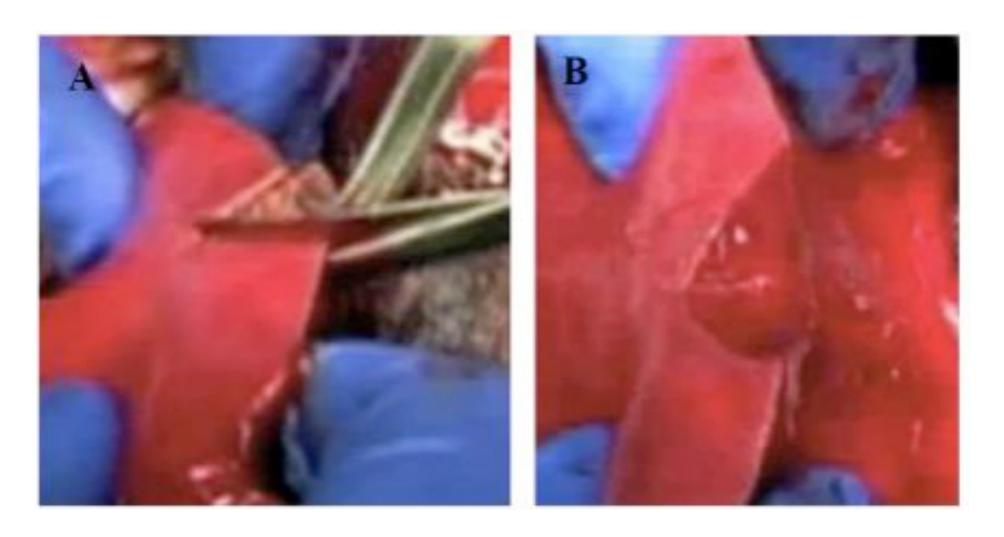# Treatment for Inaccessible Wounds

Treatment for Inaccessible Wounds

Porcine Surgical Trauma Models

- Coagulation Science
- Hemostatic Devices
- Can the wound be accessed?
- Severity of Wound
- Wound Adhesion
- Hemostasis

Noncompressible Stellate Laceration Model

Hypothermic, hemodilutional model

Wedge Resection

Lobe Resection Model

Left Lateral Lobe Transection
Noncompressible Injury Model

1: 1st PV branch to left lateral lobe
2: Lumen of transected 2nd PV branch to left lateral lobe
3: Left main branch of PV
4: Distal portion of cut 2nd branch
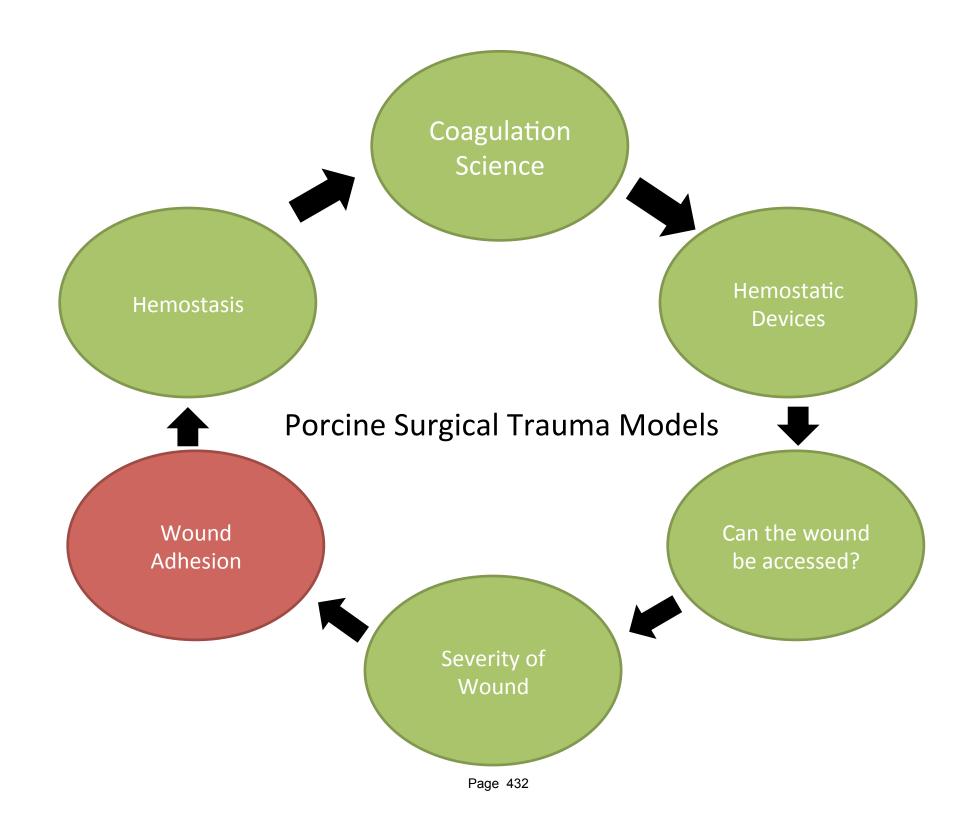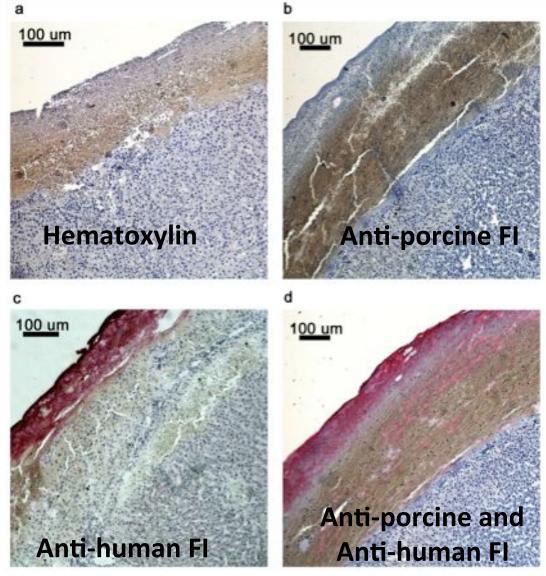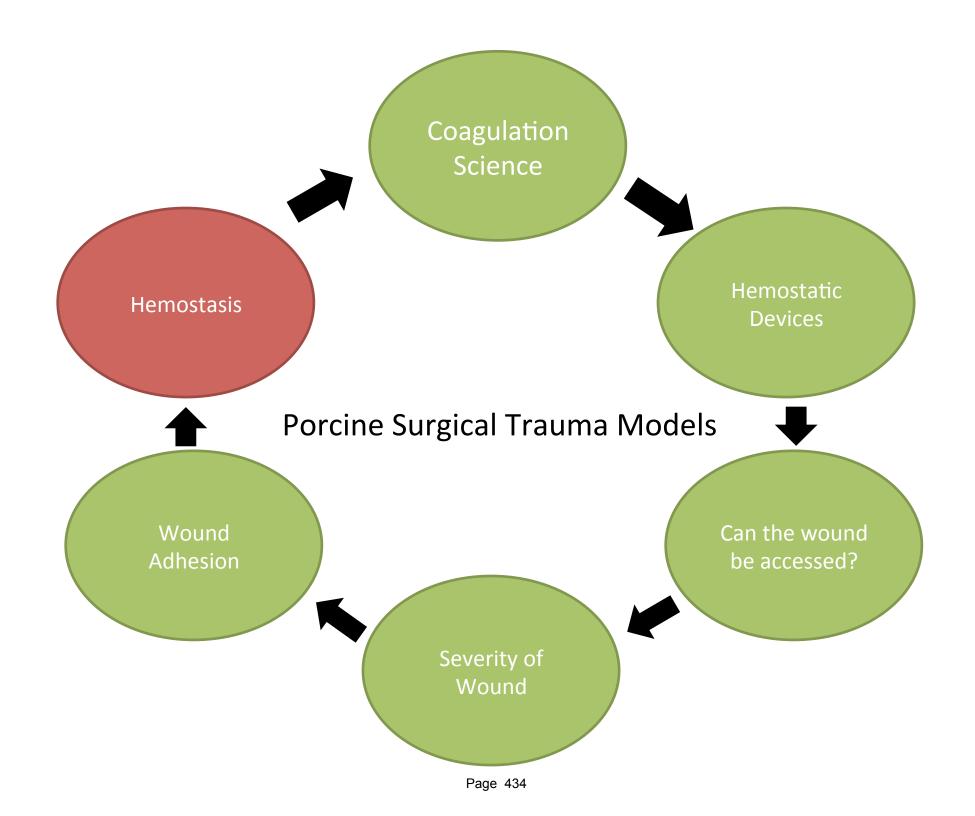5: Orifice of cut hepatic vein to left lateral lobe

Porcine Surgical Trauma Models

- Coagulation Science
- Hemostatic Devices
- Can the wound be accessed?
- Severity of Wound
- Wound Adhesion
- Hemostasis

Histology reveals the difference between 100 to 200 microns thick film of wound adherent exogenous Fibrin Sealant and native swine Fibrinogen
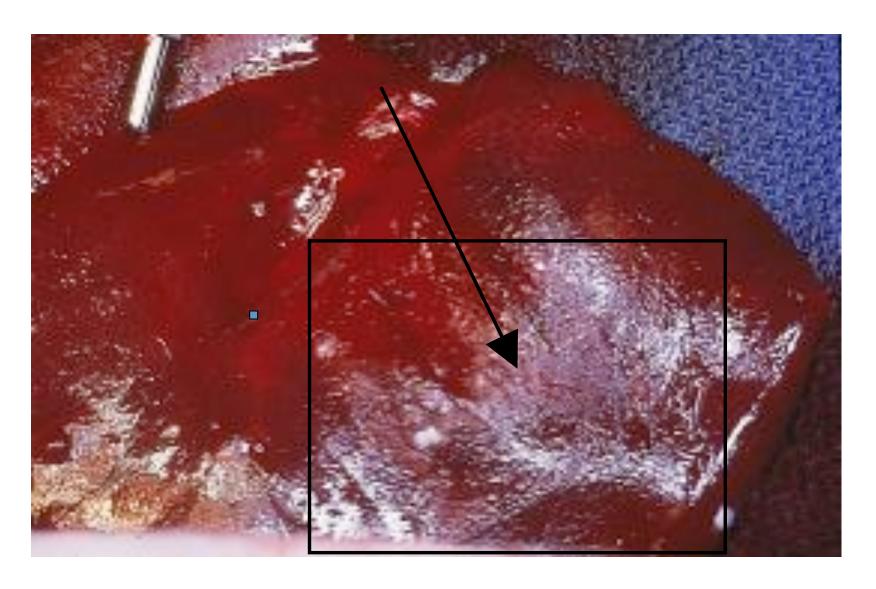
Porcine Surgical Trauma Models

Grade V+ stelate laceration:
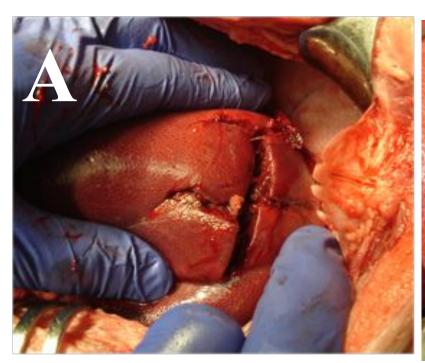# Pig liver model for exsanguinating injury

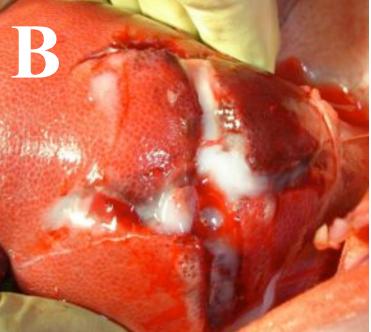# Hemostasis regained after 1 g F1 ARC-DFSD
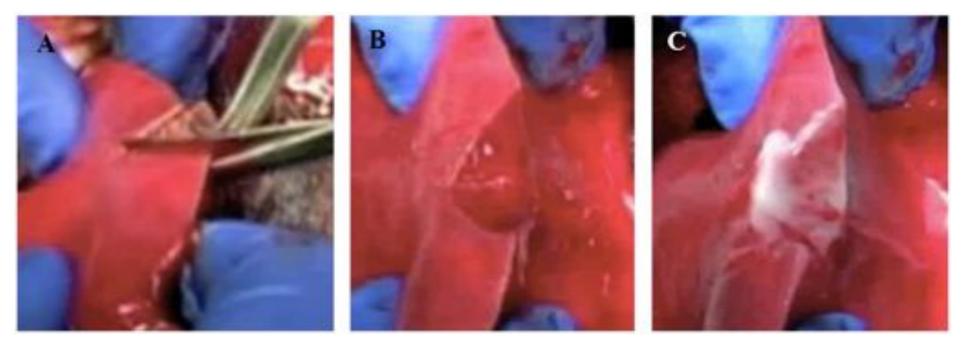
Hemostasis using 1 gram FI

Compressible pig liver single and double stellate laceration model Noncoagulopathic, Grade V+ Wound
Treated with 71 mg rFI, 17,350 U rFXIII, 846 U rFIIa delivered by a dual-syringe.

- Single laceration models received a score of 1.50 ± 0.55 (n=6)

- Double laceration models received a score of 1.67 ± 0.58 (n=3).

Wedge resection treated with LFS applied by spray-device.

Triangular-shaped wedges with bases of 1.0 cm and 0.5 to 3.0 cm heights were resected (A) resulting in profuse bleeding (B) which was treated with LFS applied by a spray-device (C). This application resulted in complete hemostasis.
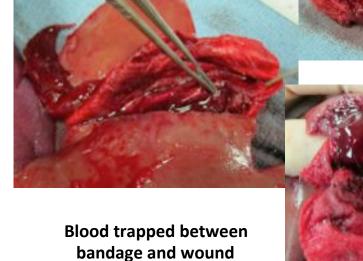
Depth (cm)	N	Hemostasis Score
0.5	11	1.18 ± 0.40
1.0	14	1.64 ± 0.50
1.5	11	2.09 ± 0.94
2.0	11	2.36 ± 0.67
2.5	10	2.90 ± 0.32
3.0	8	3.25 ± 0.46

FS on PCL (small pores)

Swine #89

Hypothermic, hemodiluted model

Loose adherence;
Not hemostatic

90 mg pdFI,
3.6 mg (25k U) rFXIIIa,
600 U rFIIa

Blood trapped between
bandage and wound

FS on PCL (large pores)

Swine #86

Hypothermic, hemodiluted model

Adhesion over most of wound; however, portion over large vessel broke free after 5 sec

90 mg pdFI,
3.6 mg (25k U) rFXIIIa,
600 U rFIIa

FS on PCL (large pores)

Swine #87

Hypothermic, hemodiluted model

**Adhesion over most of wound;
bleeding through bandage**

**90 mg pdFI,
3.6 mg (25k U) rFXIIIa,
600 U rFIIa**
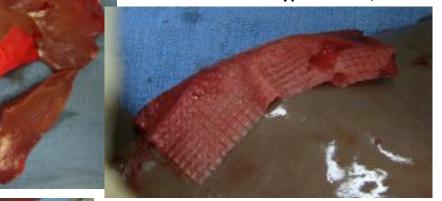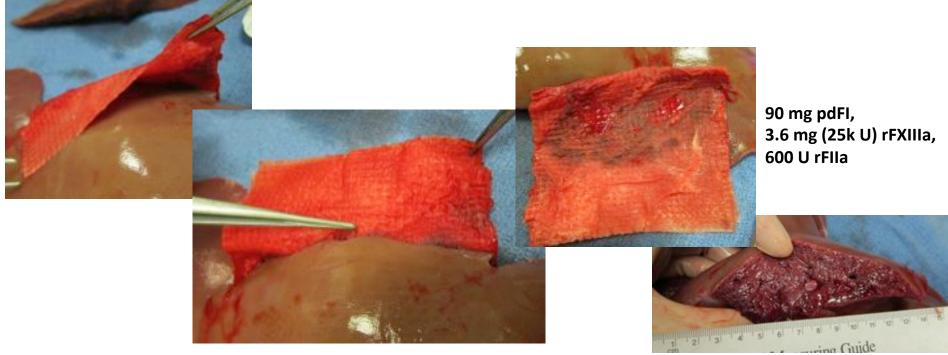
FS on PCL (midsize pores)

Swine #91
Hypothermic, hemodiluted model

Adhered to injured but not uninjured liver; bleeding noted around edge of bandage

**90 mg pdFI,
3.6 mg (25k U) rFXIIIa,
600 U rFIIa**

FS (FI:FN = 1:0.13), PLA nanofiberous bandage

Bandage adherent and hemostatic

Swine #127
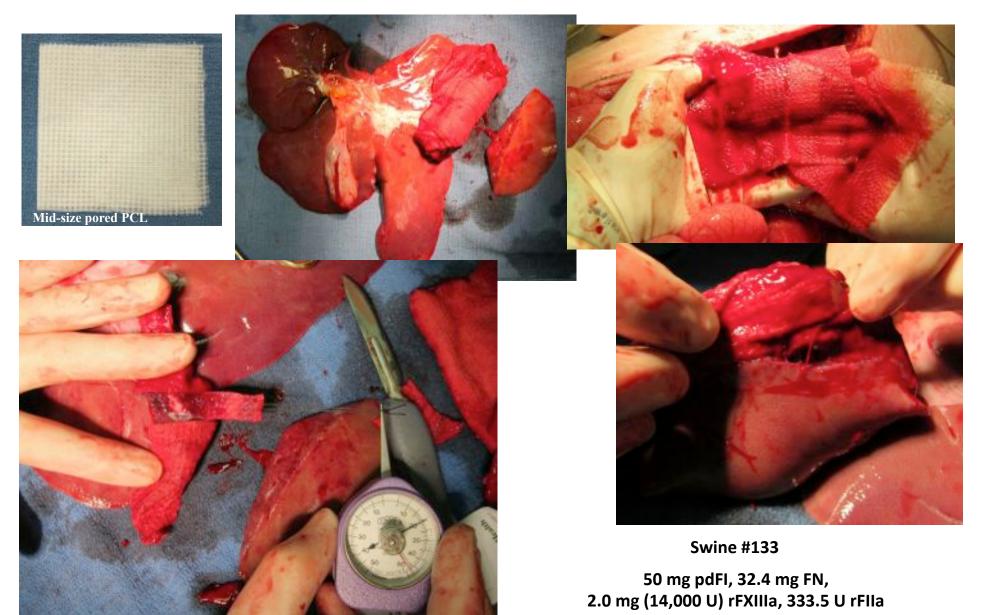
Normothermic, normovolemic model

**50 mg pdFI, 8.5 mg FN,
2.0 mg (14,000 U) rFXIIIa, 333.5 U rFIIa**

Bandage adherent

FS Coated PCL bandage

Mid-size pored PCL

Swine #133

**50 mg pdFI, 32.4 mg FN,
2.0 mg (14,000 U) rFXIIIa, 333.5 U rFIIa**

VIDEO
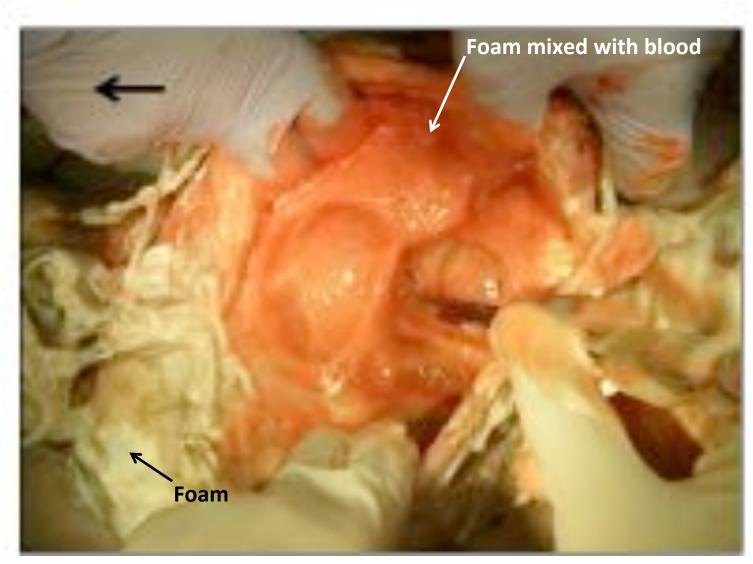
The Application of
Recombinant Fibrin Sealant
in Pig Liver Injury Models

Wedge Resection
Stellate Laceration
Lobectomy
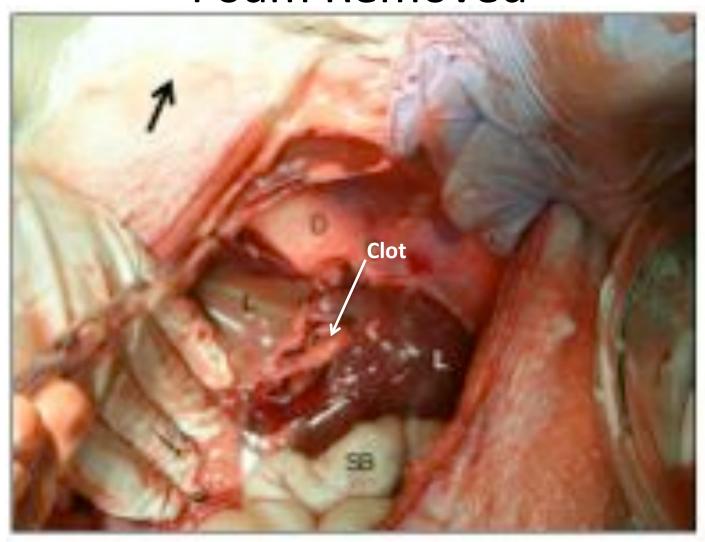
Prototype Foaming Device Application
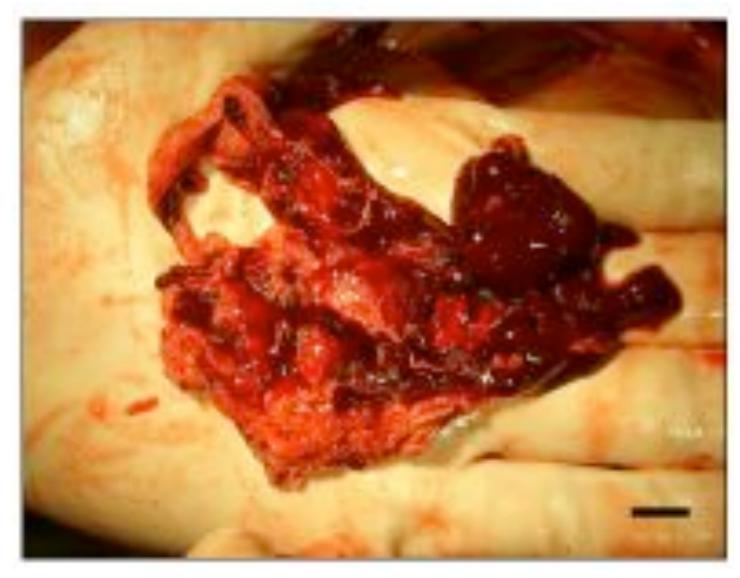
Prototype Foaming Device
Open Wound

Foam mixed with blood

Foam

Prototype Foaming Device
Foam Removed
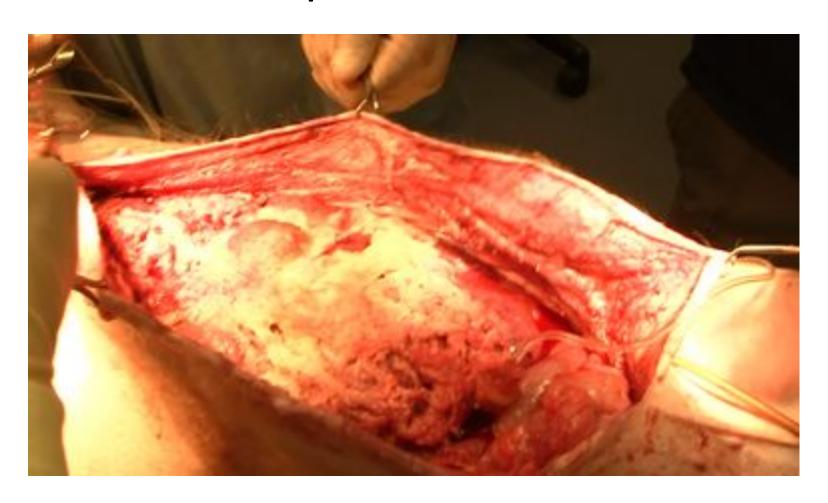
Prototype Foaming Device
Clot Removed

Long-Barreled FS Coated Foam Device Setup
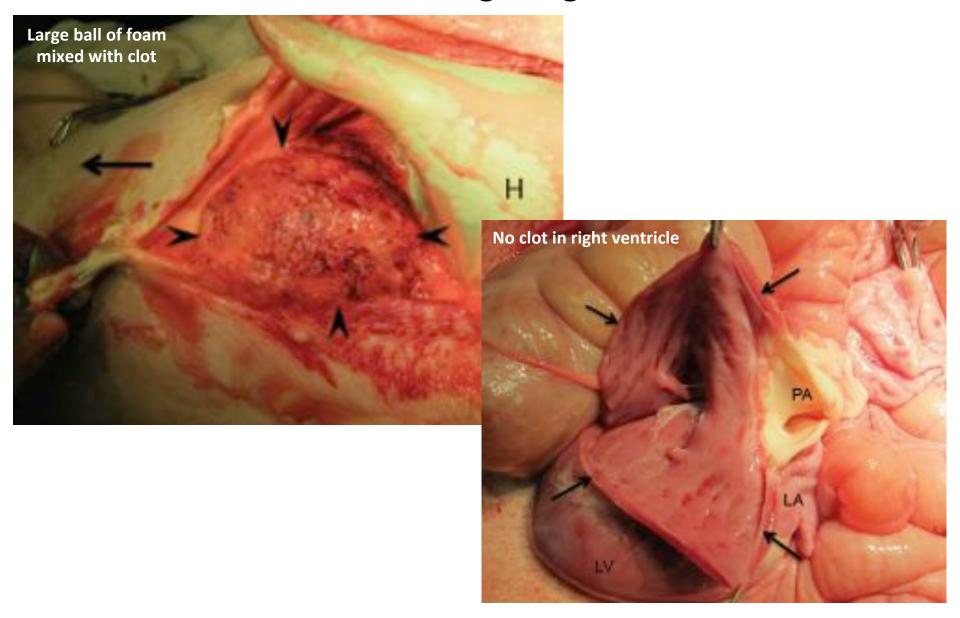
Long-Barreled FS Coated Foam Device Open Wound
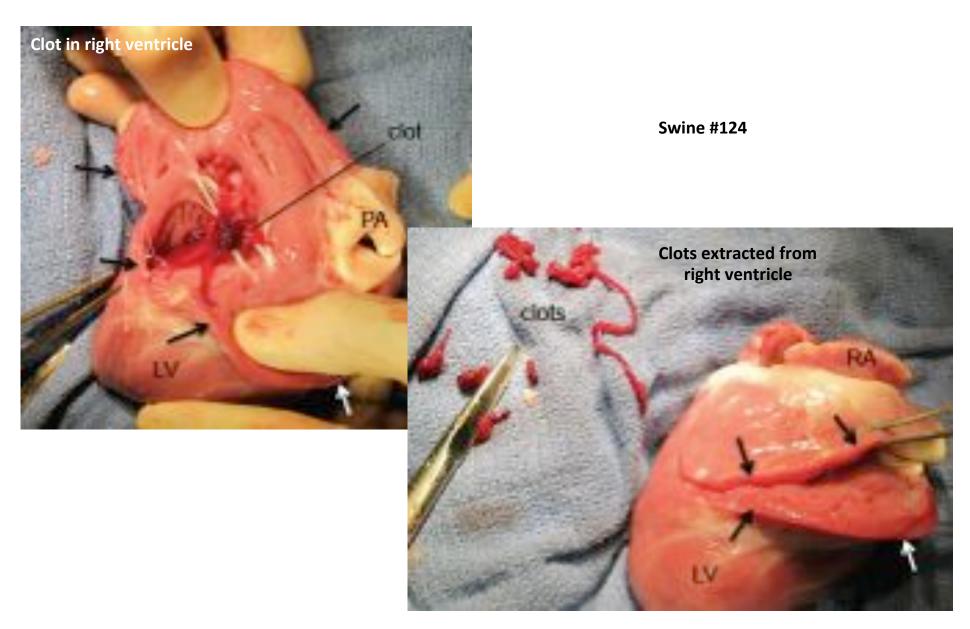
Long-Barreled FS Coated Foam Device Removed Foam
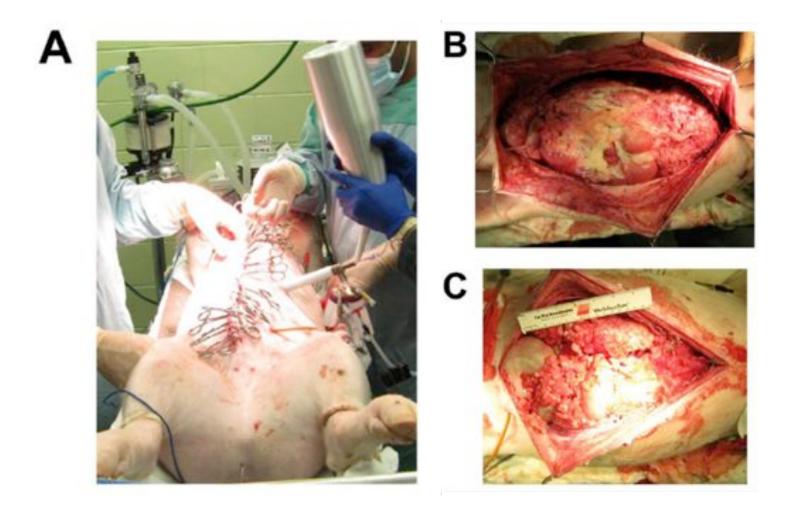
Successful Treatment using Long-Barreled FS Device

Large ball of foam mixed with clot

No clot in right ventricle

Risks: Formation of Clot inside the Heart

Clot in right ventricle

Swine #124

Clots extracted from right ventricle

Current Treatment options using FS Coated Carrier Foam

Material and Methods

- Surgeries were conducted on crossbred commercial (domestic) swine from UNL Agricultural Research and Development Center (Mead).

- The Omaha Veteran's Affairs Institutional Animal Care and Use Committee approved all procedures. All animals were treated according to the *Guide for the Care and Use of Laboratory Animals* (National Institute of Health publication 86-23, revised 1996).

- Hemostasis was measured on a scale of 1-4
  - 1: complete hemostatic;
  - 2: mostly hemostatic, minor oozing;
  - 3: partially hemostatic with prominent oozing;
  - 4: minimal effect and complete failure,

- The abdominal cavity of the anesthetized swine was opened prior to hepatic resection.

- A splenectomy was performed on the animal to allow for exsanguination similar to humans to occur after hepatic resection.

The enhanced kinetics of thromboelastic strengthening for both recombinant and plasma-derived fibrin using a monomeric recombinant Human Factor XIII A chain

Vanderslice N, Calcaterra J, Inan M, Jain V, Velander W. H.

University of Nebraska

Background

Factor XIII (FXIII) is activated by thrombin during fibrin formation releasing a dimeric, transglutaminase (FXIIIA2a) which converts fibrin into a crosslinked, viscoelastic barrier. The FXIIIA2a also covalently anchors it to exposed extracellular matrix at wound surfaces. The impact of FXIII activation kinetic lag and diffusional limitations upon achieving fibrin maximal viscoelastic strength are investigated using recombinant (r-), monomeric FXIII (r-FXIIIA1a-HIS).

Methods

An expression cassette using the cDNA for human FXIII catalytic subunit A chain was used to express r-FXIIIA1a-HIS which was purified by immobilized metal affinity chromatography (IMAC) and characterized by SDS-PAGE, Western analysis, amino terminal sequencing and high pressure size exclusion chromatography (SEC). Thromboelastography (TEG) and a chromogenic assay were used to assess crosslinking activity relative to plasma derived (pd-) human FXIII (pd-FXIII) treated by r-thrombin (r-FIIa) to form pd-FXIIIA2a. Fibrin was made with r-human fibrinogen (r-FI) purified from the milk of transgenic cows, pd-FI, and pd-FXIII depleted pd-FI using a 0.18 molar ratio of r-FIIa to pd-F1 or r-F1.

Results

Unlike previous studies, which made FXIIIA2 in yeast, the *Pichia pastoris* made monomeric FXIIIA1a-HIS with an artificial activation peptide. The rFXIIIA1a-HIS had a ≥2-fold specific activity by chromogenic assay relative to pd-FXIIIA2a. The kinetics of crosslinked fibrin formation for pdF1 concentrations typical of tissue sealants (34 mg/ml) took 1000 seconds to reach an MA=75 mm in the presence of constitutive levels of FXIII. In contrast, rFXIIIA1a-HIS treatment of pd-F1 at only 9 mg/ml took only 450 seconds to reach an MA=75 mm. A kinetic lag of fibrin intra-chain cross-linking of 2.5 minutes caused by zymogen activation was observed by SDS PAGE for reaction mixtures consisting of rFIIa, pd-FXIII, and pd-FI. In contrast, fibrin crosslinking was already present at only 1 minute in the presence of a 0.16 molar ratio of pd-FXIIIA2a or rFXIIIA1a-HIS to pd-F1.

Conclusions

The constitutively low levels of FXIII typically present in pd-F1 leads to a kinetic lag during the FXIII activation phase. The crosslinking reaction is further slowed by a diffusion limited phase which occurs on route to reaching maximal fibrin viscoelastic strength. The use of rFXIIIA1a-HIS with a kinetically favorable artificial activation peptide reduces this lag while enabling a greatly faster approach to high thromboelastic strength at lower levels of fibrinogen. The unique *in situ* activation of the rFXIII catalytic subunit to form a fully functional rFXIIIA1a-HIS by the *P. pastoris* production system can potentially provide an abundant source of transglutaminase activity for use in recombinant liquid fibrin sealants.

Figure 2. Comparison of pdFXIII and purified rFXIIIA1a-HIS

Non-Reduced SDS-PAGE

Subunit A
Subunit B

Reduced SDS-PAGE

Subunit A+B

Anti-FXIIIA Western Blot

Anti-His-tag Western Blot
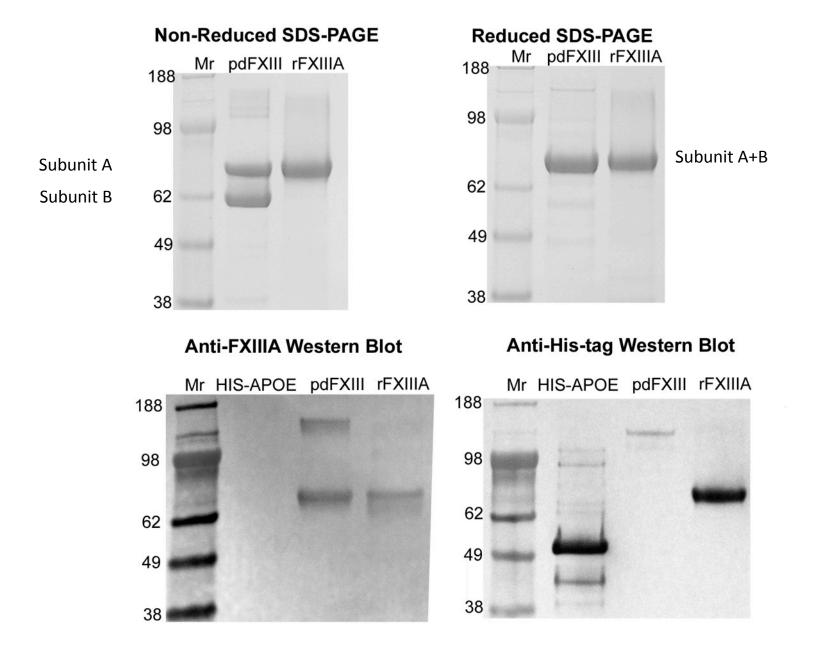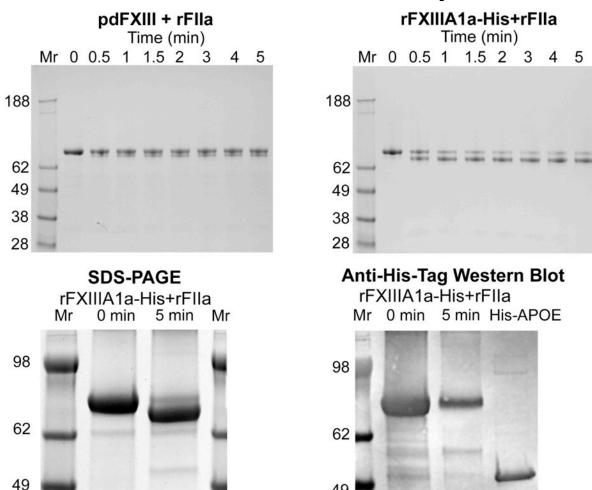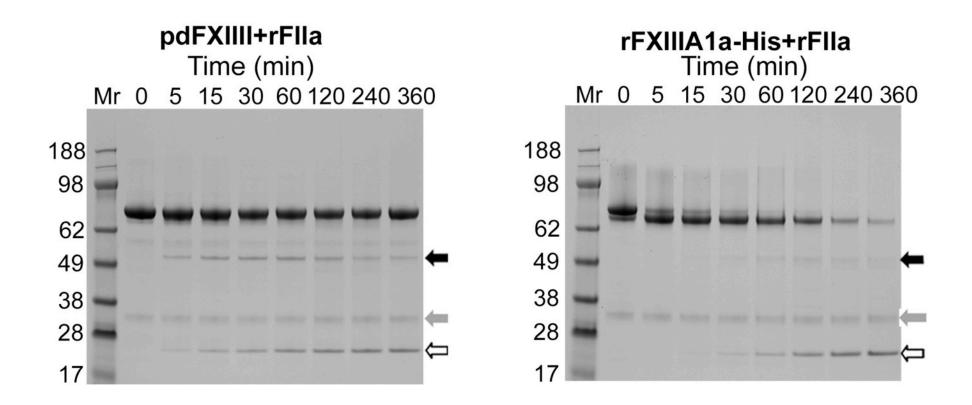
Figure 3. Time course r-FIIa activation of pd-FXIII and r-FXIIIA1a-HIS by SDS PAGE.
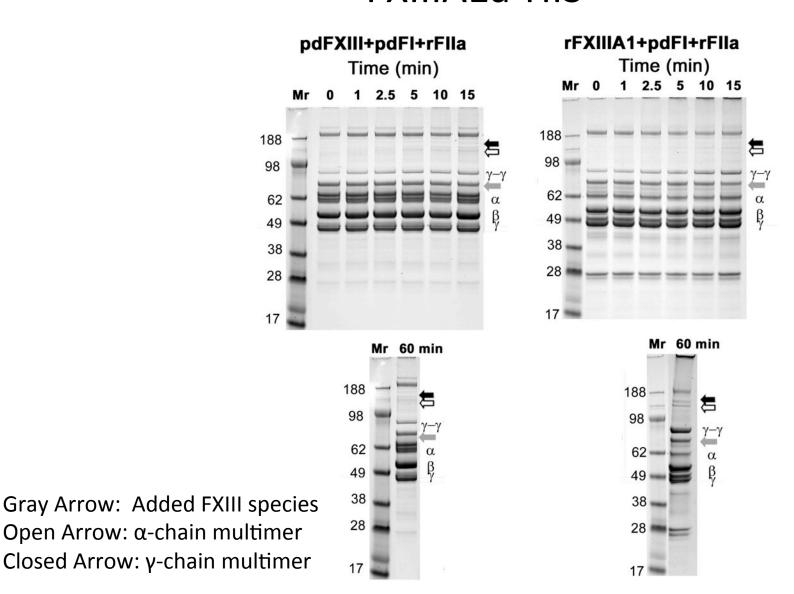
Figure 4. Time course r-FIIa proteolysis of pd-FXIII and r-FXIIIA1a-HIS by SDS PAGE
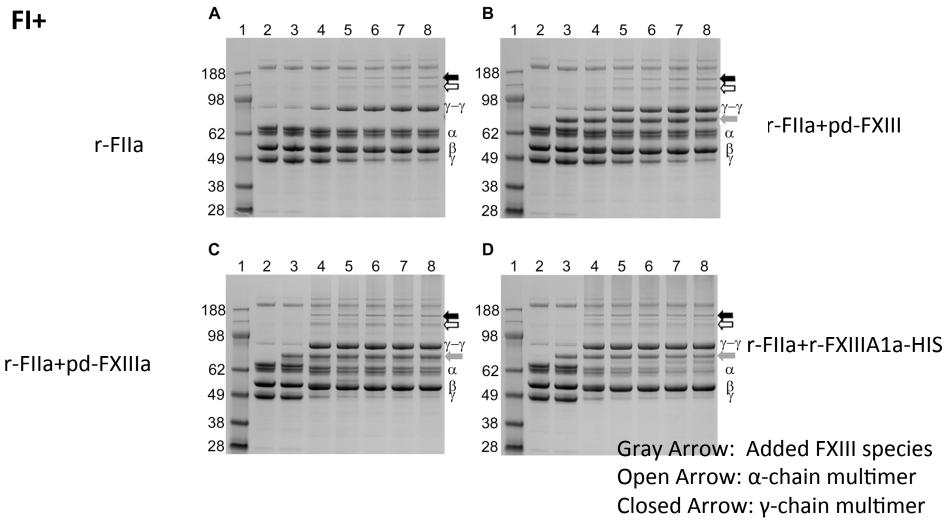
Arrows indicate degradation products

Figure 5. SDS-Page of fibrin crosslinking by r-FXIIIA1a-HIS

Gray Arrow: Added FXIII species
Open Arrow: α-chain multimer
Closed Arrow: γ-chain multimer
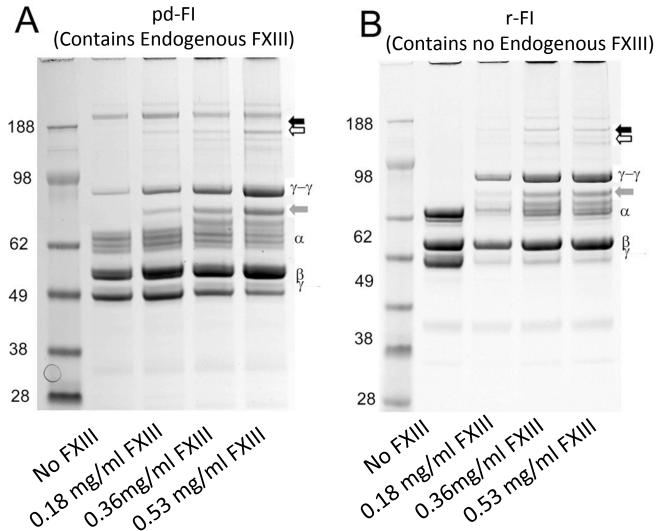
Figure 6. SDS-Page of fibrin crosslinking by r-FXIIIA1a-HIS versus zymogen pd-FXIII in the presence of purified pd-FI (0.38 mg/ml) having a typical constitutive level of FXIII activity was treated with r-FIIa (1 U/ml) to initiate fibrin formation in the absence and presence of added zymogen pd-FXIII or pd-FXIIIa having prior activation by FIIa or r-FXIIIA1a-HIS (1.1 U/ml).

Gray Arrow: Added FXIII species
Open Arrow: α-chain multimer
Closed Arrow: γ-chain multimer

Lane 1: molecular weight marker; Lane 2: pd-FI prior to r-FIIa and FXIII treatment; Lanes 3 through 8: pd- FI, r-FIIa and FXIII incubated for 0, 1, 2.5, 5, 10 and 15 minutes.
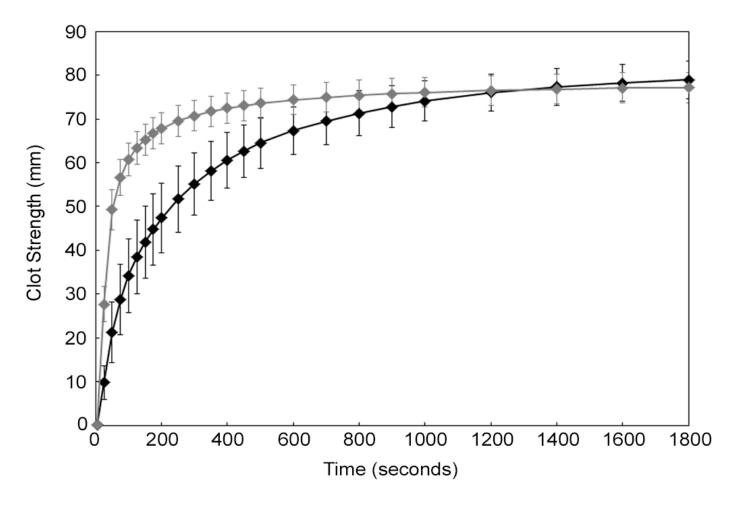
Figure 7. Dose-response SDS-Page of r-FXIIIA1a-HIS on r-FI and pd-FI

A pd-FI
(Contains Endogenous FXIII)

B r-FI
(Contains no Endogenous FXIII)

(A) pd-FI (9mg/ml) and (B) r-FI (9 mg/ml) were incubated with FIIa (0.2 mg/ml)

Gray Arrow: Added FXIII species
Open Arrow: α-chain multimer
Closed Arrow: γ-chain multimer

Figure 8. Thromboelastography acceleration and strengthening of plasma-derived biotherapeutical grade fibrin sealant by rFXIIIA1a-HIS. TEG analysis of the kinetics of clot initiation and clot strength over time for biotherapeutical grade fibrin sealant at 33.5 mg/ml (◆) and biotherapeutical grade fibrin sealant formulated at 9 mg/ml FI and 0.36 mg/ml rFXIIIA1a-HIS (◆).Data are expressed as mean +/- standard deviation. Clot was initiated with 106 U/ml of FIIa

Evidence that Extravascular Human Factor IX Influences Pharmacokinetics In Hemophilic B Dogs

Off-protocol

Nicholas C. Vanderslice*, Weijiu Xu*, Tong Gui†, Genlin Hu†, Nick Masiello‡, Paul E. Monahan†, Elizabeth P. Merricks**, Timothy C. Nichols**, Kevin E. Van Cott*, William H. Velander*

*Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln, Lincoln, Nebraska; † Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; ‡Revobiologics, Framingham MA , **Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

SSC 2014
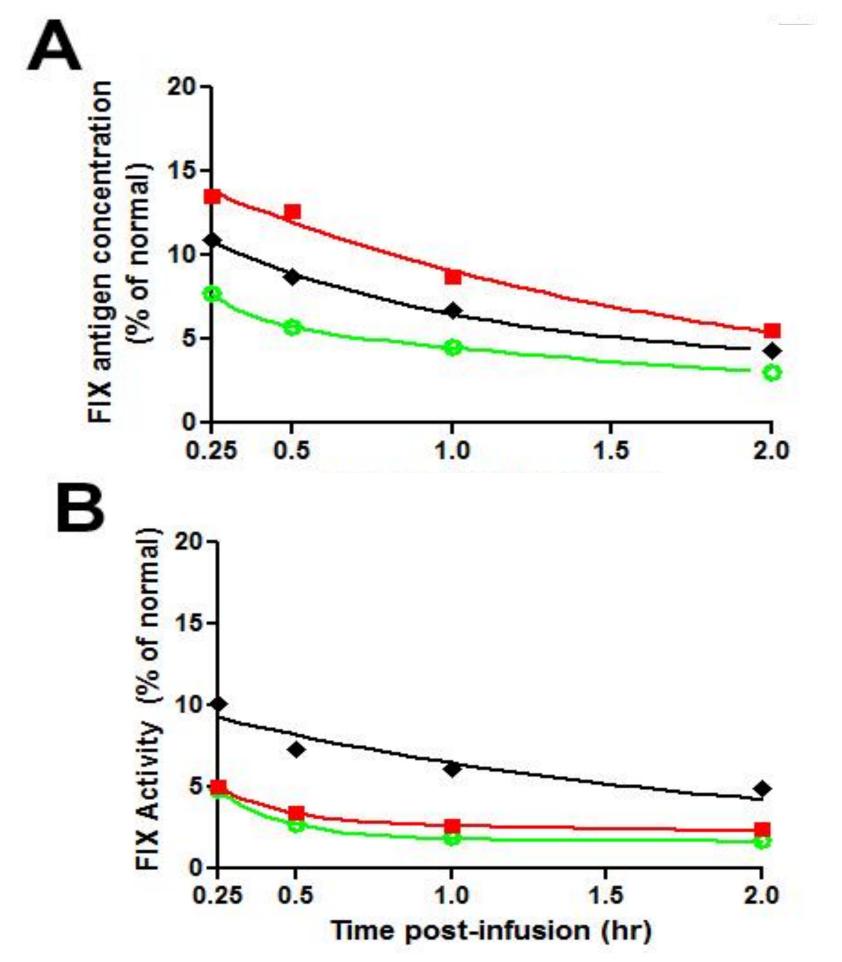
EHF02

BACKGROUND

Factor IX (FIX) is a 56 kDa glycoprotein that plays a key roll in the coagulation cascade. In the absences of active FIX, a blood clotting disorder known hemophilia B occurs. Multiple gene transgenesis was used to produce a biologically active, γ-carboxylated, recombinant human Factor IX (tg-FIX) protein with no residual propeptide. This protein was studied in hemophilic B dogs to assess safety and efficaciousness.

Table 1. Post-translational Characteristics of FIX Variants.

| Characteristic	pd-FIX[2]	r-FIX[2]	tg-FIX
Primary structure	Ala[148]/Thr[148]	Ala[148]	Thr[148]
Specific Activity (IU/mg)	181±8	178±14	155±16
Total γ-carboxyglutamic acid content			
12 of 12 Gla	100%	60%	Minor Species
11 of 12 Gla	0	35%	Major Species
10 of 12 Gla	0	5%	Major Species
<10 of 12 Gla	0	0	Minor Species
Biacore: Steady-State KD (nM)			
Ca2+-dependent mAb (SB 249417)	7.5E-8	3.8E-8	9.9E-8
Mg2+-dependent mAb (1G7)	1.7E-7	9.3E-8	3.9E-7
1G7 Affinity Chromatography (% Eluate)	100%	52.5%	34.5%
γ-carboxylated glutamic acid residues			
Gla position 1-6	All complete	All complete	All complete
Gla position 7-10	All complete	All complete	Partially complete
Gla positions 11-12	All complete	Partially complete	Partially complete
β-hydroxyaspartic acid (Asp 64)	37%	46%	40-50%
Pro-peptide content	None detected	None detected	None detected
Activated FIX	0.21% ±0.01%	0.11% ±0.01%	None detected
Tyr 155 sulfation	>90%	<15%	<15%
Ser 158 phosphorylation	>90%	<1%	70%
Sialylation (NANAmol/mol FIX)	8.8	6.5	4-5.5

MATERIALS AND METHODS

Production:
Human furin was co-expressed with human FIX in the milk of transgenic pigs using the milk promoter from murine Whey Acidic Protein. Therapeutic grade FIX was produce by CHO cells and used as a reference.

Purification and Characterization:
Purification of tg-FIX was performed using a modified version of the procedure of Lindsay et al.[1] Size exclusion chromatography was used for a polishing step for all samples used in pharmacokinetic studies to ensure no activated tg-FIX was delivered. tg-FIX was characterized by SDS-PAGE, western analysis, single-stage coagulation assay, and mass spectrometry. Mass spectrometry also assed the γ-carboxylation. tg-FIX, plasma-derived FIX (pd-FIX) and recombinant FIX produced by CHO cells (r-FIX) were also characterized using four different metal-dependent monoclonal conformational FIX Gla Domain antibodies (Two Ca2+-dependent mAbs and two Mg2+-dependent conformational mAbs (1G7 and 2C7))

Pharmacokinetic Study:
Purified plasma-derived FIX (pd-FIX) and tg-FIX were intravenously administered at 50 IU/kg to hemophilic B dogs which were monitored for 14-15 days. These studies in four hemophilic B dogs were:

• Dog O06: a single infusion of tg-FIX
• Dog O66: two successive infusions of tg-FIX where the second infusion occurred at 72 hours
• Dog O05: an infusion of tg-FIX followed by a second infusion of pd-FIX at 48 hours;
• Dog O25: an infusion of pd-FIX followed by a second infusion of tg-FIX at 48 hours

For all studies, plasma antigen, single stage clotting activity, and whole blood clotting times (WBCT) were assessed. Mean residence times (MRT) were calculated from the time course values of above assays.
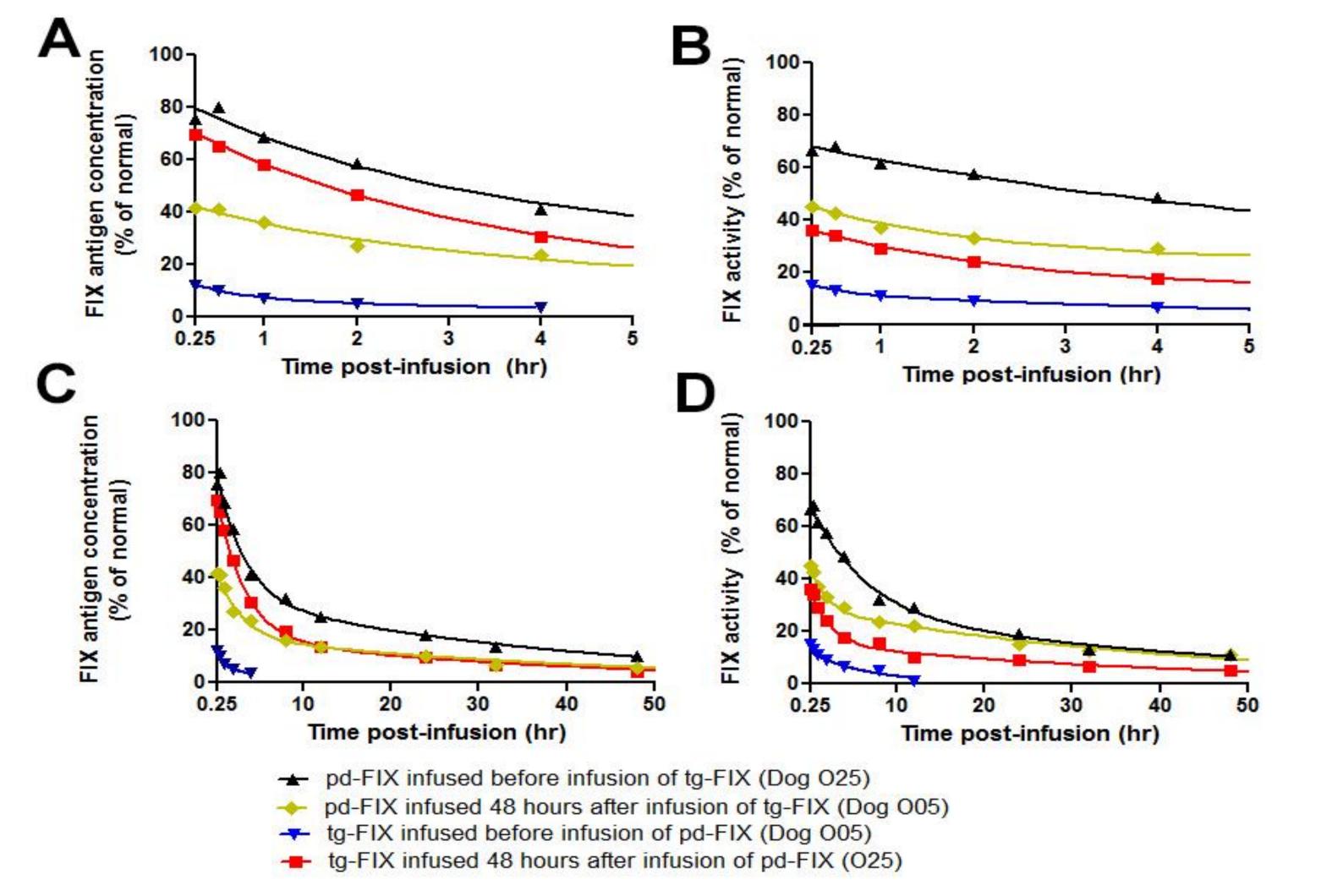
RESULTS

tg-FIX was characterized for various post-translational modifications as can be seen in Table 1. Throughout the study, all animals remained in healthy conditions and showed no sign of fever or systemic reaction to the infused materials. The baseline WBCT in all hemophilic B dogs studied was typically greater than 50 minutes (Fig. 3). In studies O06, O66, and O05, the average WBCT for the first infused on of tg-FIX was 14.5 ± 3.2 minutes for two days. Similarly, the WBCT of dog O25 after the first infusion of pd-FIX was 11.2 minutes for two days. These values are comparable to the normal value of 8-12 minutes for WBCT in normal dogs. Studies O05 and O25 showed an increase in MRT when preceded by an infusion of the other species: the MRT of pd-FIX increased 30% when preceded by tg-FIX but the MRT of the tg-FIX increased by >700% when preceded by pd-FIX (Fig. 1 and Fig. 2). Taken together, studies O05 and O25 showed evidence of differential extravascular partitioning between tg-FIX and pd-FIX due to difference in Gla domain conformation from non-processive γ-carboxylation.
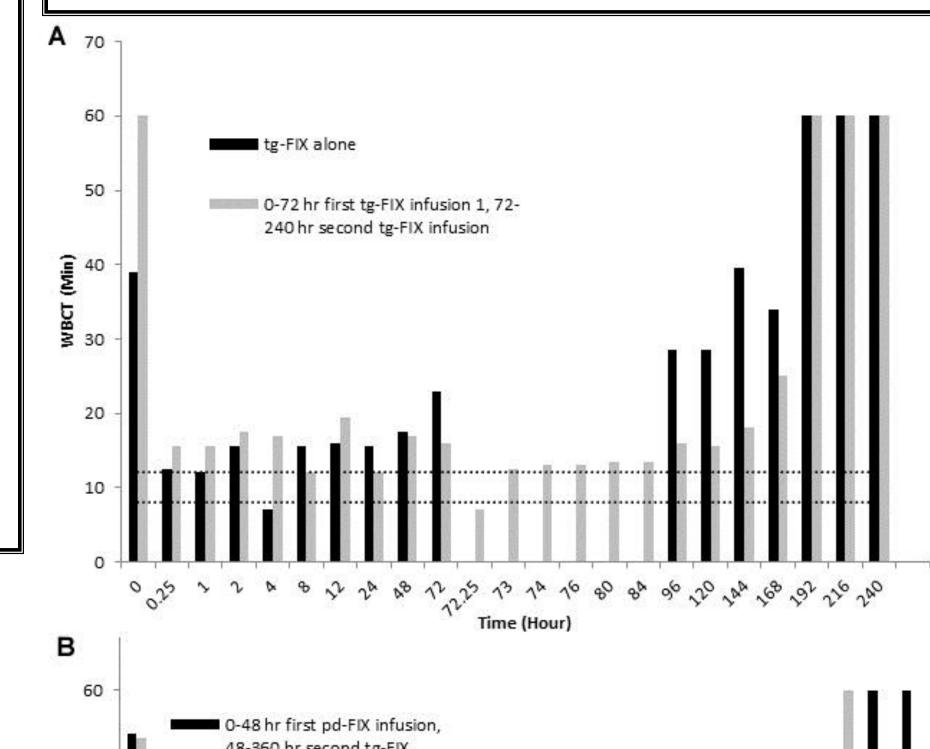
Fig. 4. Time course of WBCT in HB dogs infused with tg- and pd-FIX. A) tg-FIX infused once and tg-FIX to tg-FIX XO studies. B) pd-FIX to tg-FIX and tg-FIX to pd-FIX XO studies. Dotted lines indicate the range for WBCT in normal dogs.

tg-FIX infused once (Dog O06)
tg-FIX infused before infusion of tg-FIX (Dog O66)
tg-FIX infused 72 hours after infusion of tg-FIX (Dog O66)

Fig. 1. Effect of tg-FIX, and tg-FIX to tg-FIX XO on plasma activity and antigen levels in hemophilia B dog. FIX activity measured by (A) antigen measured by ELISA and (B) one stage clotting assay. Each line represents an individual trial of 50 U/kg tg-FIX administered to a hemophilia B dog. All animals had an antigen and activities below detectability before infusion.

pd-FIX infused before infusion of tg-FIX (Dog O25)
pd-FIX infused 48 hours after infusion of tg-FIX (Dog O05)
tg-FIX infused before infusion of pd-FIX (Dog O05)
tg-FIX infused 48 hours after infusion of pd-FIX (Dog O25)

Fig. 3. The XO pharmacokinetics of IV injected pd- and tg-FIX in Hemophilia B dog.(A, B) Recovery and (C, D) terminal antigen and activity levels for 50 U/kg XO IV delivered pd-FIX to tg-FIX and tg-FIX to pd-FIX studies in hemophilia B dog. (A, C) ELISA and (B, D) one stage clotting assay. All animals had an antigen and activities below detectability before infusion except for the 48 hour second infusion of tg-FIX following a first infusion of pd-FIX (Antigen: 10.3% Activity: 10.9%).

CONCLUSIONS

These IV infusion studies in HB dogs show that circulation residence time can be substantially lengthened by the dual infusion of FIX species with differential reservoir avidities to target potential improvements in hemostatic outcomes.

BIBLIOGRAPHY

1. Lindsay M, Gil G-C, Cadiz A, Velander WH, Zhang C, Van Cott KE. Purification of recombinant DNA-derived factor IX produced in transgenic pig milk and fractionation of active and inactive subpopulations. *Journal of Chromatography A*. 2004; **1026**: 149-57.
2. White GC, 2nd, Beebe A, Nielsen B. Recombinant factor IX. *Thromb Haemost*. 1997; **78**: 261-5.
3. Brinkhous K, Sigman J, Read M, Stewart P, McCarthy K, Timony G, Leppanen S, Rup B, Keith JJ, Garzone P. Recombinant human factor IX: replacement therapy, prophylaxis, and pharmacokinetics in canine hemophilia B. *Blood*. 1996; **88**: 2603-10.
4. Nichols T, Franck H, Franck C, De Friess N, Raymer R, Merricks E. Sensitivity of whole blood clotting time and activated partial thromboplastin time for factor IX: relevance to gene therapy and determination of post-transfusion elimination time of canine factor IX in hemophilia B dogs. *Journal of Thrombosis and Haemostasis*. 2012; **10**: 474-6.
5. Feng D, Stafford KA, Broze GJ, Stafford DW. Evidence of clinically significant extravascular stores of factor IX. *Journal of Thrombosis and Haemostasis*. 2013; **11**: 2176-8.

DEVELOPMENT OF A PORCINE MODEL OF NONCOMPRESSIBLE HEMORRHAGE

Ujwal Yanala, Dean Heimann, Chris Hansen, Tiffany Pena, Jie Chao, John Cavanaugh, William H. Velander, Gustavo Larsen, Jennifer Calcaterra, Iraklis I. Pipinos, Jason M. Johanning, Crystal Cordes, Mostafa Fatemi, Sandra Noriega, Luis Nunez, Ruben Spretz, and Wilson H. Burgess, and Mark A. Carlson

Abstract:

This research seeks to develop technologies to address the challenging problem of exsanguinating hemorrhage on the battlefield, particularly truncal (noncompressible) hemorrhage.

Exsanguination is the first or second most common cause of battlefield mortality and noncompressible hemorrhage have been especially problematic. A research priority of the US Army is to develop effective treatment for traumatic hemorrhage in subjects with noncom-pressible injuries. We believe that bleeding from complex wound topo-graphies in noncompressible, truncal injuries can be treated with a resorbable synthetic polymeric matrix combined with human clotting factors. In contrast to previous hemostatic devices, we believe our engineering approach will maximize efficacy while decreasing both polymer and biologic usage, thus providing a cost-effective device. Accordingly, we have developed the ability to engineer a variety of synthetic, resorbable proto-types at nano- and micro-scales (fiber and particulate) using minimal amounts of polymer and clotting factors. Prototype configurations range from a cotton gauze analogue to an expandable polymer. In addition, we have developed econom-ical, abundant sources of human fibrin sealant components that are kinetically faster than commercially-available sealants.

Introduction:

The first part of the study is to create a hemorrhagic model which reciprocates the bleeding problems currently existing in the battlefield. In this attempt we tried to create a bleeding model which exsan-guinates to death in one hour when no biologics are supplied.

Methods

Each animal was fasted for 12-18 hours before surgery, with free access to water. Premedication was done with a combina-tion of telazol (4.4mg/kg), ketamine (2.2 mg/kg), and xylazine (2.2 mg/kg), given as a single IM shot. An IV line was established in a marginal ear vein to provide supple-mental medication (telazol 4.4mg/kg, ketamine 2.2 mg/kg, and xylazine 2.2 mg/kg IV as needed), and euthanasia solution at the end of the procedure. The animal was masked with isoflurane (3-4%) and supplemented with oxygen (3-5 L/min) to achieve relaxation for endotracheal intubation. Once intubation was accom-plished, the animal was maintained with isoflurane (1-2%) supplemented with oxygen (1-2 L/min) throughout the proce-dure. A rectal temperature probe, pulse oximeter and EKG (cardiac) monitors were placed. The animal rested on a warming blanket. Mechanical ventilation was provided at a rate of 12-15 breaths per minute and a tidal volume of 5-10 mL/kg. End-tidal pCO2 was maintained at 35-45 mm Hg. The equipment required for these and subsequent procedures included an anesthesia machine, a ventilator, an end-tidal CO2 monitor, a rectal-temperature monitor, a warming blanket, an arterial pressure monitor, a Foley catheter, laparo-tomy and vascular surgical instruments, and a suction apparatus. Continuous vital sign data were digitally captured by a Bionet monitor.
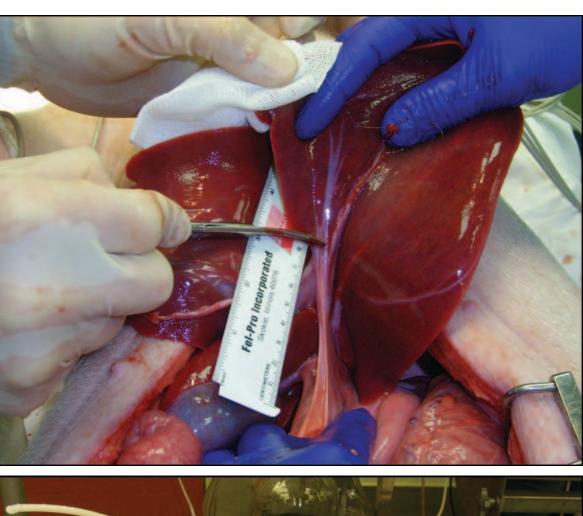

Fig. 1: Anesthesia equipment and the vital signs monitor used during operating procedure s.

Model

A carotid arterial catheter for pressure monitoring and blood sampling, and a jugular venous catheter for fluid and medication administration was placed via surgical cut down in the right neck. A midline laparotomy and splenectomy was performed to minimize autotransfusion by the contractile porcine spleen.

A calculated injury is made to the left lateral lobe of the liver involving 1 portal vein branch, 1 hepatic vein and hepatic artery branches. Control animals gets no treatment where as the test group gets the firm alginate foam directed into the abdomen away from the injury site to prevent any foam embolization into the heart. The incision is then closed and the animal is allowed to recover for 1 hour with warm saline infusions whenever the MAP falls by 20% of its pre-injured state. After 1 hour the animals are sacrificed by exsanguination.

Fig. 2: . Liver in situ of a swine model, immediately prior to injury. Tips of scissors indicate 2nd branch of left main Portal Vein to Left Lateral lobe. (Injury Site)

Fig. 3: Alginate Foam Injecting Equipment.
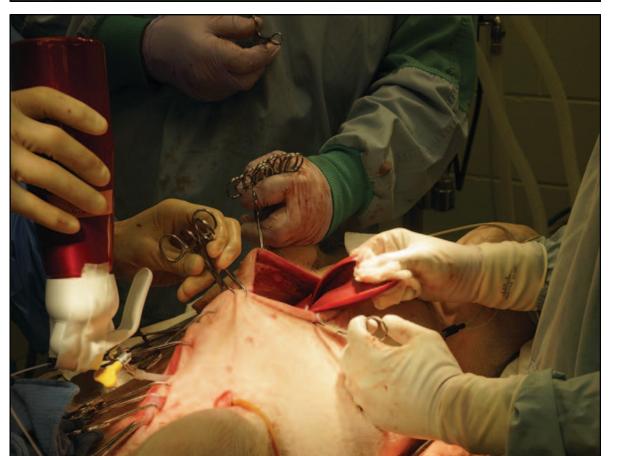
Fig. 4: Injecting mechanism. (Note the injecting site is away from the injury site). Left Lobe of Liver is also seen in the picture. Picture taken before injury.
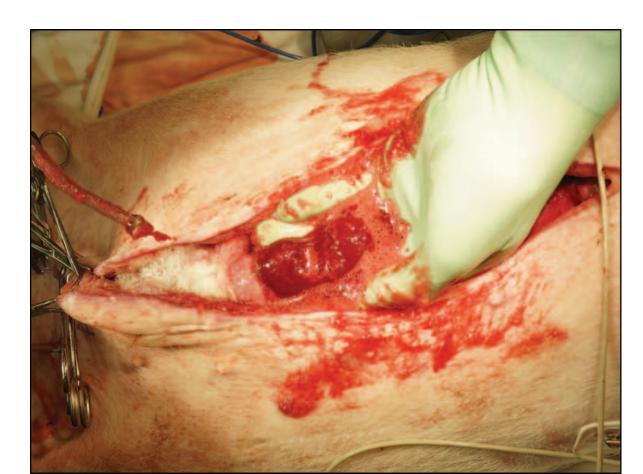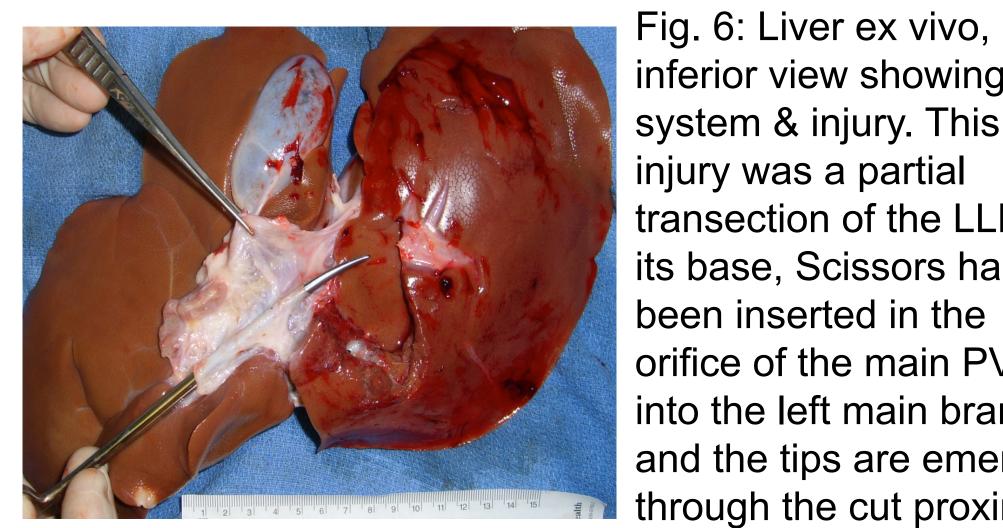
Fig. 5:. Reopening of midline incision 20 min after injury & treatment, Right is cephalad. Alginate foam in inferior portion of wound; C lot mixed with foam from superior portion of wound

Fig. 6: Liver ex vivo, inferior view showing PV system & injury. This injury was a partial transection of the LLL at its base, Scissors has been inserted in the orifice of the main PV, into the left main branch, and the tips are emerging through the cut proximal end of the 2nd branch to the LL lobe.
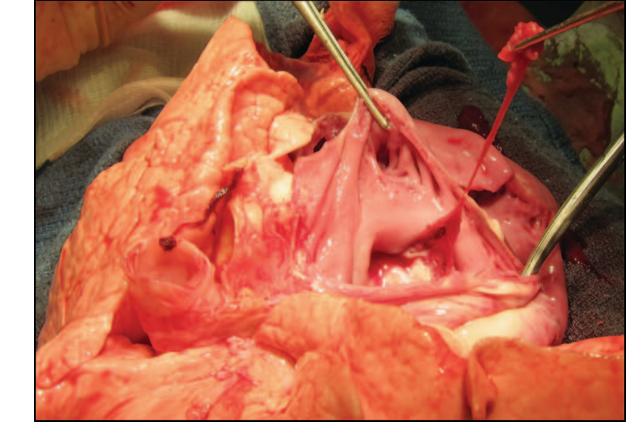
Fig. 7: Stringy red colored clot recovered from the right ventricle of the heart showing the risk of embolization when alginate foam is injected close to the injury site

Conclusion

1. The current model works as a reliable hemorrhagic subject as without any medical aid the controls die in a 30-60 minutes window.
2. The above delivery system has provided some preliminary evidence of effective drug delivery system in swine hemorrhagic models
3. The firm alginate foam delivering mechanism can be combined with other clotting factors for more effective hemostasis.

DEPARTMENT OF THE ARMY
US ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
ARMY RESEARCH OFFICE
P.O. BOX 12211
RESEARCH TRIANGLE PARK, NC 27709-2211
June 30, 2014

REPLY TO
ATTENTION OF

Life Sciences Division
Subject: Proposal No. 65184-LS-RIP

Dr. Mark Carlson
Surgery
University of Nebraska Medical Center
983280 Nebraska Medical Center
Omaha, NE 68198-3280

Dear Dr. Carlson:

   Technical evaluation of your proposal entitled "Tensiometer for bandage-wound adhesion studies" has been completed. We are pleased to inform you that our recommendation to accept your proposal has been approved. The RDECOM Acquisition Center will contact the business office of your institution to negotiate an official financial agreement.

   This letter is only for your information. It does not constitute legal commitment, nor does it authorize you to make expenditures prior to the award of a formal contract. Communications involving contractual matters must be sent to the RDECOM Acquisition Center by the appropriate official from your institution. Communications of a scientific nature should be directed to me (email: stephanie.a.mcelhinny.civ@mail.mil, voice: (919) 549-4240); in particular, all changes affecting execution of technical aspects of the award need to be communicated to me in a timely manner.

   If your research involves use of humans or animals, you are hereby notified that you may not conduct any experiments involving human or animal use until you have received Institutional Review Board (IRB) approval from the U.S. Army. IRB approval from your own institution is also required, but is not sufficient. It is highly recommended that you contact the Army Office of Research Protections immediately to initiate this approval process. The POC for human use is HRPO, email usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil, or voicemail at (301)619-2165. The POC for animal use is Ms. Nina Cisar at (301)619-6694.

   Annual funding of increments or options for your agreement are contingent upon receipt of required reports. These reporting requirements are described in the Reporting Instructions Section of the ARO website (http://www.aro.army.mil).

   Congratulations on the acceptance of your proposal; we look forward to a mutually satisfactory research program. Please do not hesitate to contact me if you have any questions.

Sincerely,

Stephanie A. McElhinny
Life Sciences Division

Copy Furnished:
Primary Administrative Representative

AWARD/CONTRACT	1. THIS CONTRACT IS A RATED ORDER UNDER DPAS (15 CFR 700)		RATING	PAGE OF PAGES
				1 5

2. CONTRACT (Proc. Inst. Ident.) NO. W911NF-14-1-0430	3. EFFECTIVE DATE 01 Aug 2014	4. REQUISITION/PURCHASE REQUEST/PROJECT NO. 0010553035

5. ISSUED BY CODE W911NF	6. ADMINISTERED BY (If other than Item 5) CODE N66018
US ARMY ACC-APG-RTP W911NF 4300 S. MIAMI BLVD DURHAM NC 27703	ONRRO SAN DIEGO 140 SYLVESTER ROAD BLDG 140 ROOM 218 SAN DIEGO CA 92106-3521

7. NAME AND ADDRESS OF CONTRACTOR (No., street, city, county, state and zip code)	8. DELIVERY [ ] FOB ORIGIN [X] OTHER (See below)
UNIVERSITY OF NEBRASKA 987835 NEBRASKA MEDICAL CENTER OMAHA NE 68198-6810	9. DISCOUNT FOR PROMPT PAYMENT Net 0 days

10. SUBMIT INVOICES **2**	ITEM
(4 copies unless otherwise specified) TO THE ADDRESS SHOWN IN:	

CODE 1PPD6	FACILITY CODE

11. SHIP TO/MARK FOR CODE W36QYT	12. PAYMENT WILL BE MADE BY CODE HQ0490
TRANSPORTATION OFFICE - W36QYT PR PROP BK ACCT DURHAM PO BOX 12211 RESEARCH TRIANGLE PARK NC 27709-2211	DFAS INDIANAPOLIS-GFEBS 8899 EAST 56TH STREET DEPT. 3800 INDIANAPOLIS IN 46249-3800

13. AUTHORITY FOR USING OTHER THAN FULL AND OPEN COMPETITION: [ ] 10 U.S.C. 2304(c)( ) [ ] 41 U.S.C. 253(c)( )	14. ACCOUNTING AND APPROPRIATION DATA **See Schedule**

15A. ITEM NO.	15B. SUPPLIES/ SERVICES	15C. QUANTITY	15D. UNIT	15E. UNIT PRICE	15F. AMOUNT
	SEE SCHEDULE				

15G. TOTAL AMOUNT OF CONTRACT	$58,020.00

16. TABLE OF CONTENTS

(X)	SEC.	DESCRIPTION	PAGE(S)	(X)	SEC.	DESCRIPTION	PAGE(S)
		PART I - THE SCHEDULE				**PART II - CONTRACT CLAUSES**	
X	A	SOLICITATION/ CONTRACT FORM			I	CONTRACT CLAUSES	
	B	SUPPLIES OR SERVICES AND PRICES/ COSTS				**PART III – LIST OF DOCUMENTS, EXHIBITS AND OTHER ATTACH.**	
	C	DESCRIPTION/ SPECS./ WORK STATEMENT			J	LIST OF ATTACHMENTS	
	D	PACKAGING AND MARKING				**PART IV - REPRESENTATIONS AND INSTRUCTIONS**	
	E	INSPECTION AND ACCEPTANCE			K	REPRESENTATIONS, CERTIFICATIONS AND OTHER STATEMENTS OF OFFERORS	
	F	DELIVERIES OR PERFORMANCE					
	G	CONTRACT ADMINISTRATION DATA			L	INSTRS., CONDS., AND NOTICES TO OFFERORS	
	H	SPECIAL CONTRACT REQUIREMENTS			M	EVALUATION FACTORS FOR AWARD	

CONTRACTING OFFICER WILL COMPLETE ITEM 17 (SEALED-BID OR NEGOTIATED PROCUREMENT) OR 18 (SEALED-BID PROCUREMENT) AS APPLICABLE

17. [ ] CONTRACTOR'S NEGOTIATED AGREEMENT Contractor is required to sign this document and return _____ copies to issuing office.) Contractor agrees to furnish and deliver all items or perform all the services set forth or otherwise identified above and on any continuation sheets for the consideration stated herein. The rights and obligations of the parties to this contract shall be subject to and governed by the following documents: (a) this award/contract, (b) the solicitation, if any, and (c) such provisions, representations, certifications, and specifications, as are attached or incorporated by reference herein. (Attachments are listed herein.)	18. [X] SEALED-BID AWARD (Contractor is not required to sign this document.) Your bid on Solicitation Number _____ including the additions or changes made by you which additions or changes are set forth in full above, is hereby accepted as to the terms listed above and on any continuation sheets. This award consummates the contract which consists of the following documents: (a) the Government's solicitation and your bid, and (b) this award/contract. No further contractual document is necessary. (Block 18 should be checked only when awarding a sealed-bid contract.)

19A. NAME AND TITLE OF SIGNER (Type or print)	20A. NAME OF CONTRACTING OFFICER
	SUSAN P. HILL / GRANTS/CONTRACTING OFFICER TEL: 919-549-4338 EMAIL: susan.p.hill.civ@mail.mil

19B. NAME OF CONTRACTOR	19C. DATE SIGNED	20B. UNITED STATES OF AMERICA	20C. DATE SIGNED
BY _____ (Signature of person authorized to sign)		BY *Susan P. Hill* (Signature of Contracting Officer)	14-Jul-2014

AUTHORIZED FOR LOCAL REPRODUCTION

Previous edition is NOT usable

STANDARD FORM 26 (REV. 5/2011)
Prescribed by GSA – FAR (48 CFR) 53.214(a)

Section B - Supplies or Services and Prices

ITEM NO	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT PRICE	AMOUNT
0001		1	Each	$58,020.00	$58,020.00
	INSTRUMENTATION AWARD ---COST				
	FOB: Destination				
	PURCHASE REQUEST NUMBER: 0010553035				
				ESTIMATED COST	$58,020.00
	ACRN AA				$58,020.00
	CIN: GFEBS001055303500001				

Section G - Contract Administration Data

ACCOUNTING AND APPROPRIATION DATA

AA: 02120142015204000006616112550030002618R.0009959.26.4   6100.9000021001
COST CODE: A60FJ
AMOUNT: $58,020.00
CIN GFEBS001055303500001: $58,020.00

This grant will provide funding for the acquisition of research instrumentation listed in the Recipient's proposal titled "Tensiometer for bandage-wound adhesion studies" dated 21 October 2013, which is incorporated by reference. The estimated cost of the research instrumentation is set forth in the budget, attached as Exhibit A.

The Principal Investigator is Dr. Mark Carlson, (402) 559-4581, email: macarlso@unmc.edu

ARO Grants Officer's Representative is Dr. Stephanie A. McElhinny, (919) 549-4240, email: stephanie.a.mcelhinny.civ@mail.mil

This grant is issued pursuant to the authority of 10 U.S.C. 2358.

CFDA No.: 12.431.

Period of Performance: 1 August 2014 – 31 July 2015

AWARD AMOUNT: $58,020
FUNDED AMOUNT: $58,020

 1. TERMS AND CONDITIONS: This grant is subject to the Research Terms and Conditions dated JUNE 2011, to the U.S. Army Research Office Agency-Specific Requirements dated 1 JUN 2009, and to any special considerations as contained in the below mentioned Article titled "Special Terms and Conditions." These terms and conditions are incorporated by reference with the same force and effect as if they were given in full text. The full text of the terms and conditions may be accessed electronically at http://www.aro.army.mil/forms/forms2.htm.

2. PAYMENTS:

   A. Pursuant to the Debt Collection Improvement Act of 1996 (Public Law 104-134) and 32 CFR Chapter I, Subchapter C, it is a Government wide requirement to use electronic funds transfer (EFT) for the payment of any grant for which an application or proposal was submitted or renewed on or after July 26, 1996. This policy is mandatory unless the Recipient has obtained a waiver by submitting to the head of the pertinent Federal agency a certification that it has neither an account with a financial institution nor an authorized payment agent.

   B. Grant payments to the Recipient shall be made by reimbursement. The Recipient may participate in the OCNR/NRFCWASH Web Based-Invoicing System called "PayWeb." All payments will be made via funds transfer per the payment information obtained from the System for Award Management (SAM) and the Contractor Electronic Funds Transfer (CEFT) database. The Recipient will submit requests for payment to the Administrative Grants Officer using OCNR/NRFCWASH PayWeb/EDI/EFT bill paying network system.

   C. Wide Area Work Flow (WAWF) has been designated as the Department of Defense (DoD) standard for electronic invoicing and payment. To facilitate this effort for universities and nonprofit organizations, DoD

established PayWeb as one initial entry point to WAWF. The Recipient may submit all invoices under this grant using the standard PayWeb process. PayWeb will feed these payment requests to WAWF and perform approvals and certifications in the actual WAWF system.

    D. To comply with the above initiative, the Recipient should register in WAWF and have your CAGE Code activated. Your Electronic Business (EB) Point of Contact is responsible for activating the CAGE Code on WAWF by calling 1-866-618-5988. Once the Recipient is activated, the EB will self-register on the WAWF (https://wawf.eb.mil) and follow the instructions for a group administrator. The ONR Regional Office will assist in this process. The ONR Regional Office listed in Block 6 of the Award Page (SF 26) is your contact for all matters relating to the use of WAWF and PayWeb.

    E. An alternate method of reimbursement is the submission of a SF 270, Request for Advance or Reimbursement. The Recipient shall submit requests for payment no more frequently than monthly and the requests shall be submitted to the Office of Naval Research (ONR) Regional Office identified in Block 6 of the SF 26. Payment will be made by the payment office identified in Block 12 of the SF 26.

    F. Payment inquiries shall be made to the payment office identified in Block 12 of the SF 26. The toll free number is 1-888-332-7366, Option #2.

3. ADMINISTRATION: This grant is administered by the Grantor and the Office of Naval Research (ONR) regional office identified in Block 6 of the SF 26. See Article No. 18 titled "Delegation of Administration Duties," contained in the above referenced U.S. Army Research Office Agency-Specific Requirements for the identity of the administration duties delegated to the ONR.

4. FUNDING INCREMENTS AND OPTIONS: The Grantor's obligation to provide funding for increments and/or options is pursuant to Article No. 15 titled "Option to Renew" and Article No. 23 titled "Incremental Funding Actions," contained in the above referenced U.S. Army Research Office Agency-Specific Requirements.

5. REPORTING REQUIREMENTS:

    A. FINANCIAL REPORTING: Federal Financial Report (SF 425): Annual and Final Reports

Reporting period end dates fall on the end of the calendar year for annual reports (12/31) and the end date of the grant project or period for the final report. Annual reports are due 30 days after the reporting period end date, and the final report is due 90 days after the end date of the grant.

All financial reports shall be submitted to the ONR regional office identified in Block 6 of the SF 26. Copies of the forms and instructions may be found on the Internet at http://www.aro.army.mil/forms/forms2.htm.

Note: Due to current DOD funds' disbursement deficiencies, recipients are required to invoice for expenditures on a monthly basis. Disbursement deficiencies may lead to a reduction or delay in the PI's yearly budget allocation. Recipients shall email the ARO GOR a copy of each monthly invoice.

    B. TECHNICAL REPORTING: A final report shall be submitted within 90 days following the end of the specified performance period, or any authorized extension thereto, listing all items of equipment actually acquired by name, manufacturer where possible, cost, and a description of any special circumstances regarding the acquisition of the equipment. The report will also include a concise summary of the research projects on which the equipment has been or will be used, including support of (a) the research work described in the proposal and (b) other research work of interest to DoD. See ARO Form 18, Reporting Instructions, Final Progress Report, found on ARO's Homepage at http://www.aro.army.mil/forms/forms2.htm for submission instructions.

6. UNOBLIGATED BALANCES AND LIMIT OF FEDERAL LIABILITY: The ARO does not consider this award to be a continuation of any previous project.

7. ACCEPTANCE OF GRANT: The Recipient is not required to countersign this grant document. However, the Recipient agrees to the conditions specified in the grant, the above referenced Research Terms and Conditions, and the U.S. Army Research Office Agency-Specific Requirements unless notice of disagreement is furnished to the Grants Officer within fifteen (15) days after the Grants Officer's signature. In case of disagreement, the Recipient shall not assess the grant any costs of research unless such disagreement(s) is resolved.

8. SPECIAL TERMS AND CONDITIONS:

   A. Substitute equipment having the same function, features, and capabilities as that specified in Exhibit A may be purchased without further authorization. Other equipment may not be substituted without first obtaining the written approval of the Grants Officer.

   B. Title to all equipment shall vest with the recipient.

EXHIBIT A -- Budget

UNIVERSITY OF Nebraska
Medical Center
NEBRASKA'S HEALTH SCIENCE CENTER

COPY

SPONSORED PROGRAMS ADMINISTRATION

September 22, 2014

Tonya Catterton
Thomas D. Morris, Inc.
4001 Millender Mill Road
Reisterstown, MD 21136

RE: Subaward Agreement between University of Nebraska Medical Center (UNMC) and Thomas D. Morris, Inc. titled "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound" Agreement # 35-5360-2010-001.

Dear Ms. Catterton,

This is official notification that the University of Nebraska Medical Center is formally terminating the agreement listed above. This is in accordance with Article 24 of the agreement allowing either party to terminate, for any reason, with a 30 day written notice. No deliverables were generated on this project and no invoices were received.

For any questions about the project, please contact UNMC Principal Investigator Dr. Mark Carlson at macarlson@unmc.edu or 402-559-4300. For any questions about the agreement, please contact spadmin@unmc.edu or 402-559-7494.

Sincerely,

Deborah K. Vetter
Director, Sponsored Programs Administration
University of Nebraska Medical Center

Subject: RE: Subcontract cancellation (11-1-0836) (UNCLASSIFIED)

Date: Wednesday, October 8, 2014 10:30:17 AM CT

From: Clement, Jessica E CTR USARMY MEDCOM CDMRP (US)

To: Carlson, Mark A

CC: William Velander, Gustavo Larsen, Dubick, Michael A CIV USARMY MEDCOM AISR (US), Prorok, Greg D, McCoy, Matthew T, Ciccarello, Brigit M CTR USARMY MEDCOM CDMRP (US)

Classification: UNCLASSIFIED
Caveats: NONE

Dr. Carlson,

It seems as though there will be no changes in the SOW, only adding additional animals. In order to process the budget change for the subcontract termination, please provide an updated budget showing how the remaining funds will be utilized. I have attached an example cost variance budget. Please send the updated budget along with the request justification to the Grants Specialist, Ms. Elena Howell, elena.g.howell.civ@mail.mil, and copy the GOR, Dr. Dubick, and me on the request. Dr. Dubick will also need to check over the request for approval. Once the request is processed at contracting, you will receive either an official MOD or email approval for the rebudget. Then you may implement the budget changes.

It would be helpful to have a copy of the cancellation letter for our records.

Also, please make sure to go through the appropriate channels to amend the ACURO protocol, so that the changes may be implemented. I have cc'd Ms. Brigit Ciccarello if you have any regulatory compliance questions.

Please let me know if you have any questions.

Thanks,
Jessica

Jessica Clement, MS
Project Officer, Program Analyst
United States Army Medical Research and Material Command (USAMRMC)
Congressionally Directed Medical Research Programs (CDMRP)
1053 Patchel St.
Fort Detrick, MD 21702
Jessica.e.clement.ctr@mail.mil
(301) 619-4047

From: Clement, Jessica E CTR USARMY MEDCOM CDMRP (US)
Sent: Monday, October 06, 2014 12:29 PM
To: 'Carlson, Mark A'
Cc: William Velander; Gustavo Larsen; Dubick, Michael A CIV USARMY MEDCOM AISR (US); Prorok, Greg D; McCoy, Matthew T
Subject: RE: Subcontract cancellation (UNCLASSIFIED)

Classification: UNCLASSIFIED
Caveats: NONE

Mark,

Thank you for the notification. I am inquiring with the Grants Specialist for guidance in processing this request. I will let you know when I receive more information.

Best,
Jessica

Jessica Clement, MS
Project Officer, Program Analyst
United States Army Medical Research and Material Command (USAMRMC)
Congressionally Directed Medical Research Programs (CDMRP)
1053 Patchel St.
Fort Detrick, MD 21702
Jessica.e.clement.ctr@mail.mil
(301) 619-4047

From: Carlson, Mark A [mailto:macarlso@unmc.edu]
Sent: Friday, October 03, 2014 4:54 PM
To: Clement, Jessica E CTR USARMY MEDCOM CDMRP (US)
Cc: William Velander; Gustavo Larsen; Dubick, Michael A CIV USARMY MEDCOM AISR (US); Prorok, Greg D; McCoy, Matthew T
Subject: Subcontract cancellation

Award No.: W81XWH-11-1-0836
Project Title: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"

Jessica,

Drs. Velander, Larsen, and I have decided to cancel our subcontract with the Thomas D. Morris Institute (TDMI). This subcontract was supposed to have been active in FY4 of the award (Oct 1 2014 through Sep 30 2015). If you would like a copy of the official letter of cancellation, please contact Matthew McCoy, UNMC Sponsored Programs Administration (mtmccoy@unmc.edu, copied on this email).

The purpose of this cancelled subcontract was for TDMI to perform independent studies of our foam therapy for noncompressible hemorrhage. After much discussion, Drs. Velander, Larsen and I have concluded that in the remaining year (FY4) of this award, we should focus on further development and refinement of the foam therapy at our institution.

We would like to be permitted to redirect the FY4 funds that were supposed to have gone to TDMI ($213,057, per the 2010 award application) toward work performed at our institution, specifically, for further development and refinement of the foam therapy. This work would involve porcine models of hemostasis, as covered under our current ACURO protocol. We would need to amend this protocol to increase the number of swine, but otherwise we would not be performing any experimentation that would fall outside of the project's Statement of Work.

Please let us know if this will be workable and how we could proceed.

Thank you,
Mark

Mark A. Carlson, MD, FACS
Professor

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Department of Genetics, Cell Biology, and Anatomy
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Classification: UNCLASSIFIED
Caveats: NONE

Classification: UNCLASSIFIED
Caveats: NONE

Treatment of Hepatic Resection in Swine using Novel Delivery Methods for Fibrin Sealant

Nicholas C. Vanderslice*, Jennifer Calcaterra*, Ayman Ismail*, Mostafa Fatemi**, Chris Hansen***, Crystal Cordes***, Dean Heimann***,Ujwal Yanala***, Ruben Spretz **, Gustavo Larsen*, Luis Nuñez**, Mark A. Carlson***, William H. Velander*

* Chemical and Biomolecular Engineering, University of Nebraska; ** LNK CHEMsolutions, LLC; *** Department of Surgery, University of Nebraska Medical Center

Appendix Q
Off-protocol

SWINE IN BIOMEDICAL RESEARCH 2014
INTERNATIONAL CONFERENCE
July 6-8, 2014. Raleigh, NC

BACKGROUND

Fibrinogen (FI), factor XIII (FXIII), and thrombin (FIIa) are three components of liquid fibrin sealant (LFS). The FDA has approved LFS for regaining hemostasis in the case of uncontrolled bleeding. Several novel prototype systems have been designed to apply LFS derived from recombinant (r) and human plasma sources. A porcine hepatic resection model was developed as a tool to study LFS hemostatic devices. Several prototype application platforms were designed to deliver LFS to the wound site. These device designs include: a spray device capable of delivering a mist of LFS to the wound site, a biodegradable electrospun polymer gauze treated with LFS before application to the liver, and a carrier foam device capable of creating an abdominal tamponade effect while delivering LFS to the site of the injury. Including control surgeries, over 200 combined swine surgeries have been performed to date using these devices. The pig serves as an ideal surgical trauma model for developing hemostatic devices where the wound-LFS interface can be studied with immunohistochemistry that discerns endogenous pig clot from exogenous human fibrin.

MATERIALS AND METHODS

Surgeries were conducted on crossbred commercial (domestic) swine from UNL Agricultural Research and Development Center (Mead). The Omaha Veteran's Affairs Institutional Animal Care and Use Committee approved all procedures. All animals were treated according to the *Guide for the Care and Use of Laboratory Animals* (National Institute of Health publication 86-23, revised 1996). Hemostasis was measured on a scale of 1-4 (1: complete hemostatic; 2: mostly hemostatic, minor oozing; 3: partially hemostatic with prominent oozing; 4: minimal effect and complete failure), The abdominal cavity of the anesthetized swine was opened prior to hepatic resection. A splenectomy was performed on the animal to allow for exsanguination similar to humans to occur after hepatic resection.
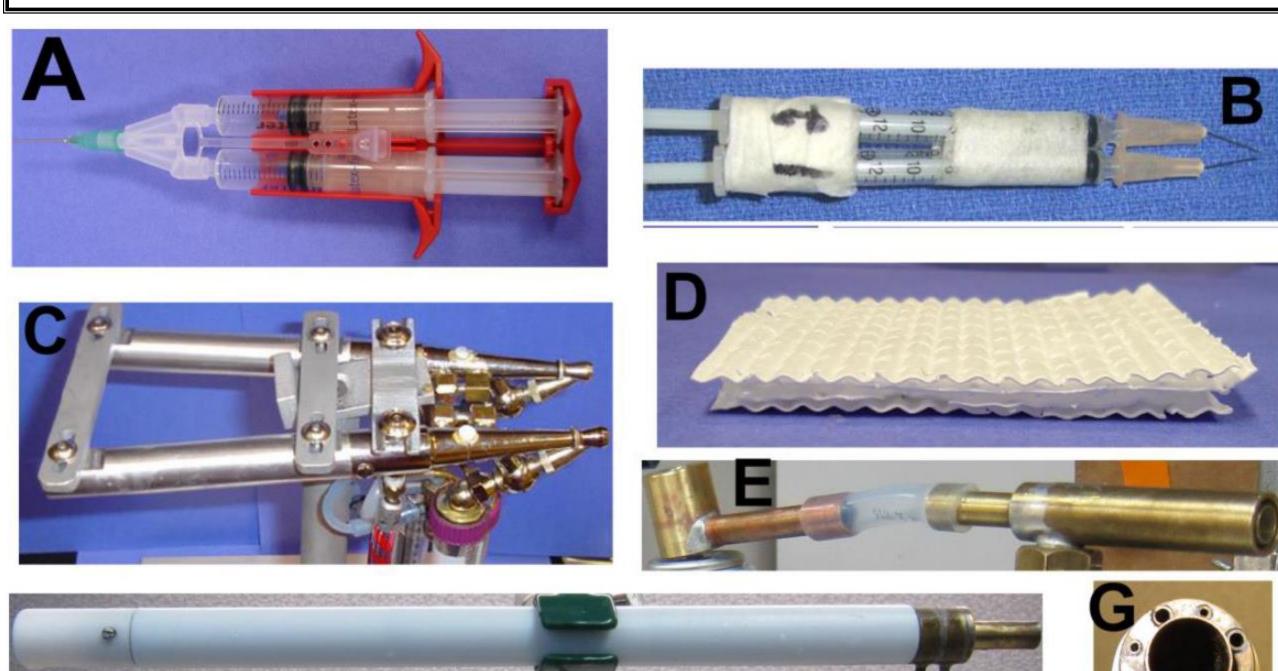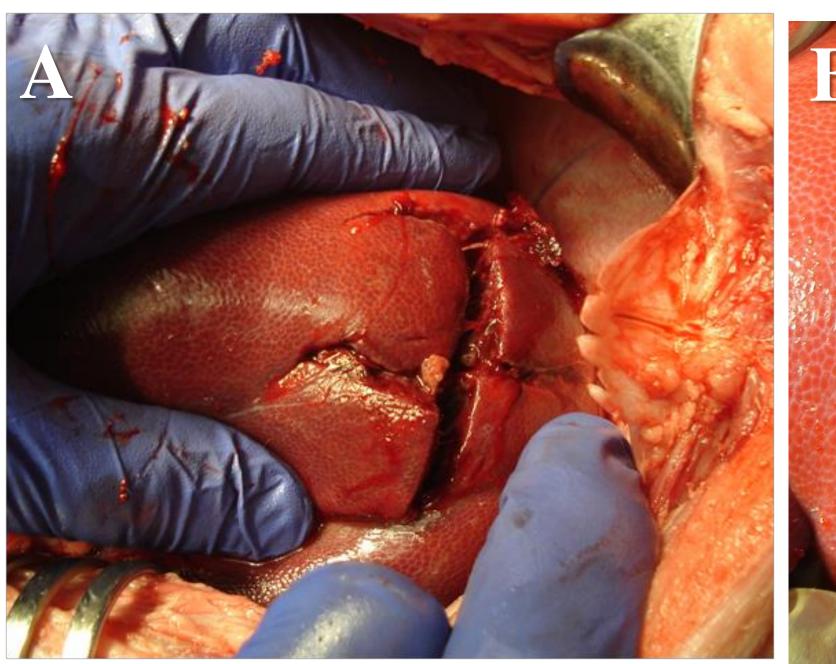

Figure 1. Fibrin Sealant Devices studied in Swine Surgeries. Proprietary dual-chamber syringe device (Duploject, Baxter) for administration of the commercial FS [2]. (B) Improvised double syringe assembly for dministration of FS [2]. (C) Dual-airbrush FS device designed to mix fibrin sealant as a mist in transport (D) Perforated, corrugated PLA bandage. (E) Device designed to coat carrier foam with FS. (E) Multi-channel device designed to coat carrier foam with FS. (G) Front of multi-channel device. Large channels are designed to carry highly viscous fibrinogen at the same rate as the low viscosity thrombin and Factor XIII in the smaller channels.
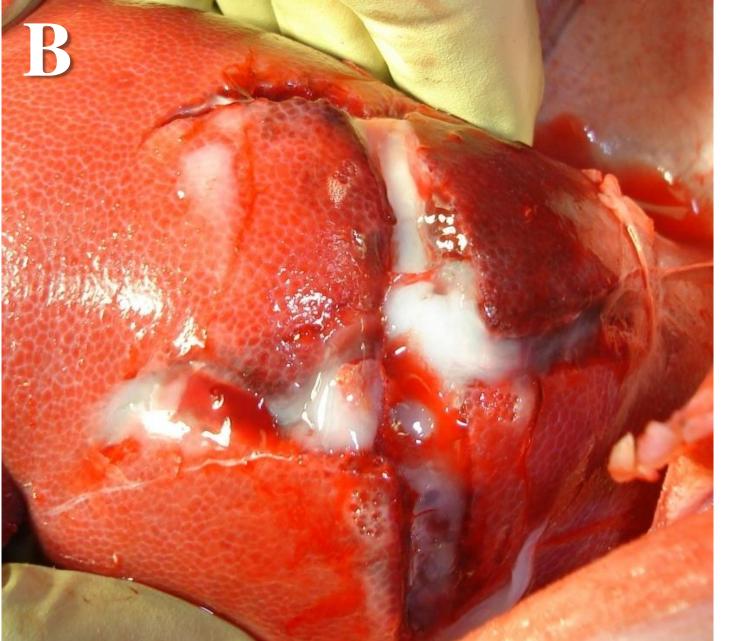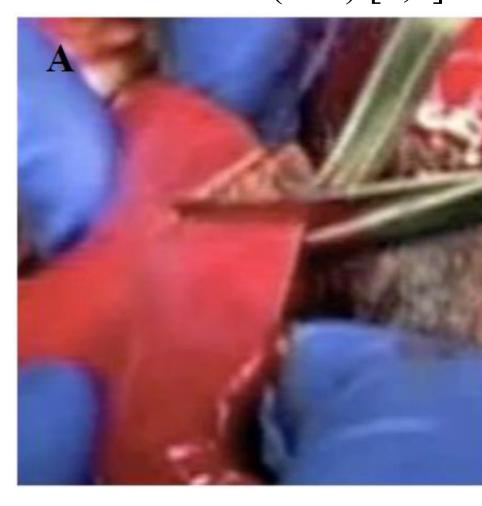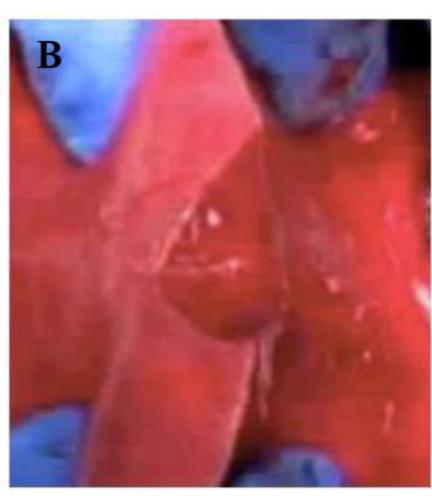
RESULTS

Figure 2. Compressible pig liver single and double stellate laceration model (noncoagulopathic, grade V+ wound) treated with 71 mg rFI, 17,350 U rFXIII, 846 U rFIIa delivered by a dual-syringe. Single laceration models received a score of 1.50 ± 0.55 (n=6) while double laceration models received a score of 1.67 ± 0.58 (n=3) [1,2].
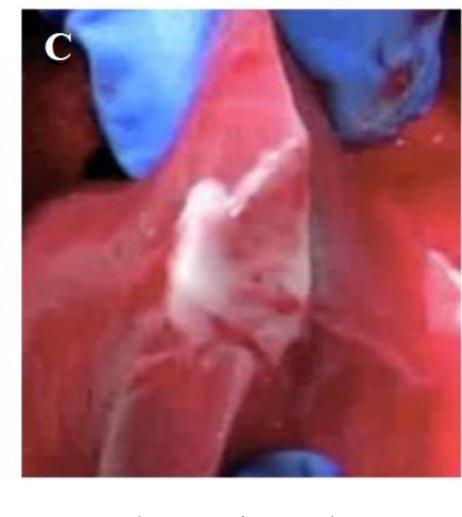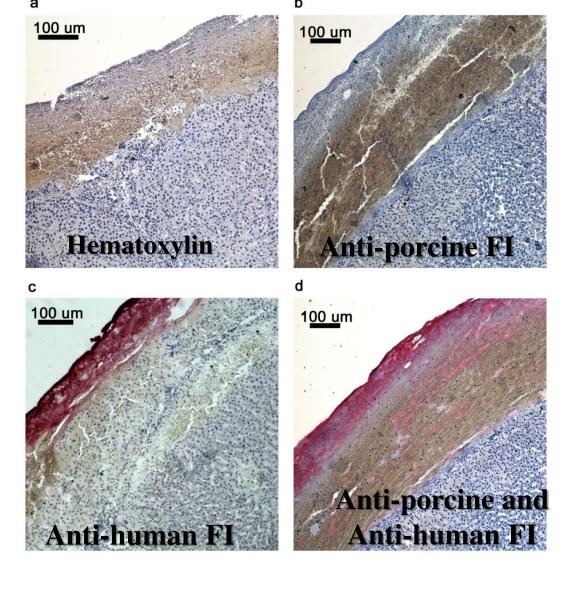

Figure 3. Wedge resection treated with LFS applied by spray-device. Triangular-shaped wedges with bases of 1.0 cm and 0.5 to 3.0 cm heights were resected (A) resulting in profuse bleeding (B) which was treated with LFS applied by a spray-device (C). This application resulted in complete hemostasis.

Table 3. Average hemostasis scores for excision depths after application of LFS by the spray device. Data presented as average ± standard deviation.

Depth (cm)	N	Hemostasis Score
0.5	11	1.18 ± 0.40
1.0	14	1.64 ± 0.50
1.5	11	2.09 ± 0.94
2.0	11	2.36 ± 0.67
2.5	10	2.90 ± 0.32
3.0	8	3.25 ± 0.46

Figure 4. Fibrin made from rFI adheres to wounded tissue. Tissue from a wedge-shaped excision along a lobar liver edge in swine treated with the rFI sealant was stained with hematoxylin in the absence of antibodies (a), and with anti-porcine FI antibody (b), anti-human FI antibody (c) and anti-porcine and anti-human FI antibodies (d) [1,2].
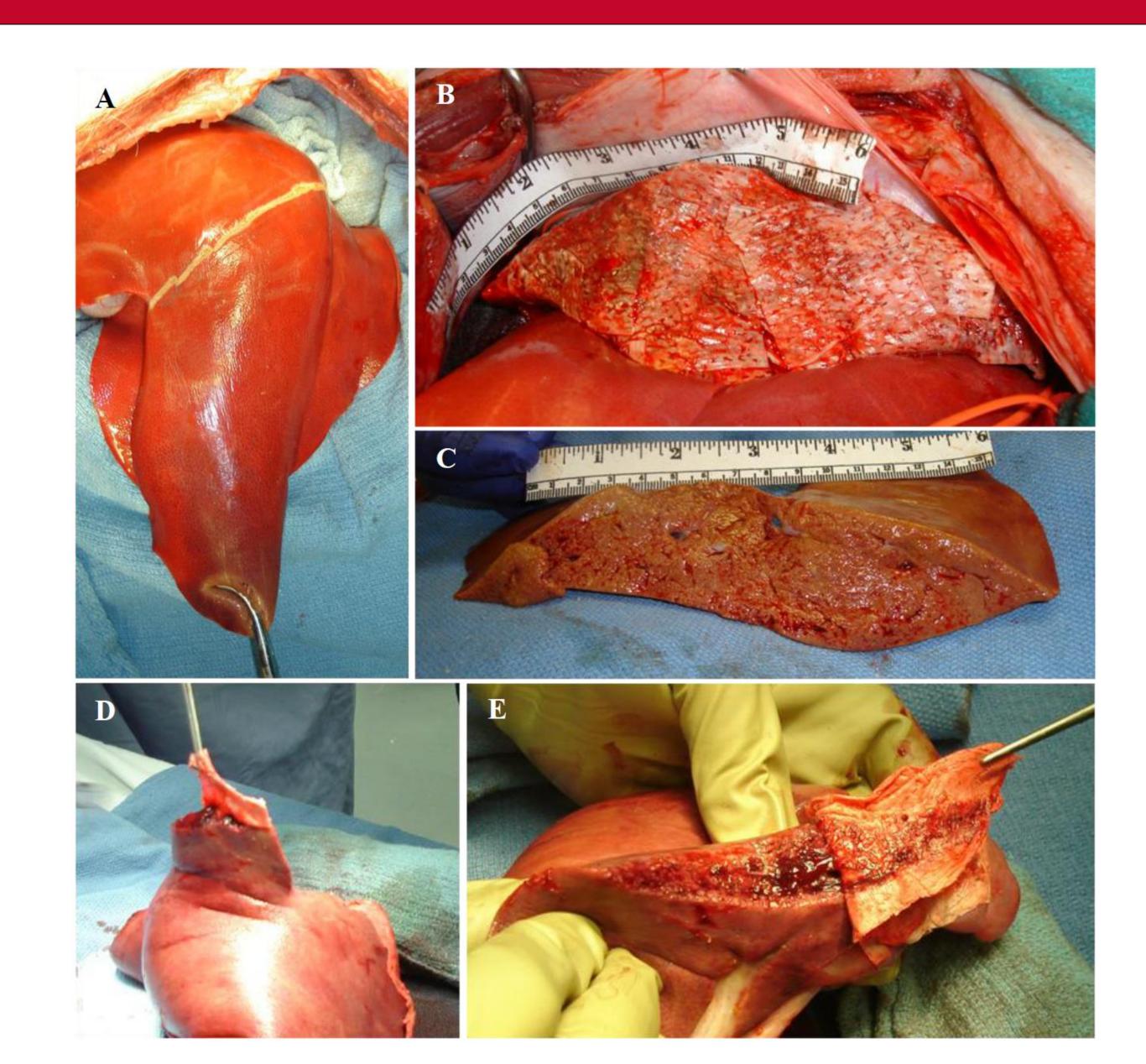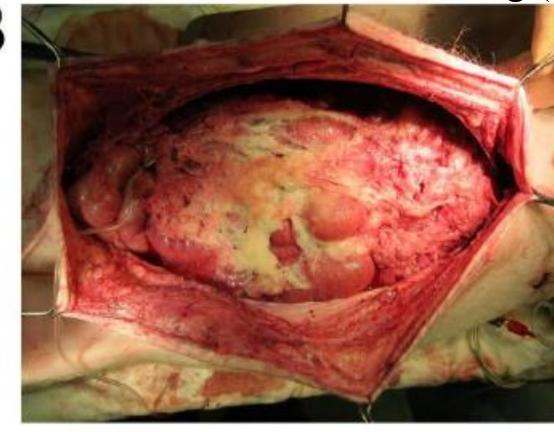

Figure 5. Treatment of a large hepatic resection with nanofiberous PDLLA coated with LFS. The planned resection, consisting of ~25% of the liver mass, was marked by a cauterization tool (A). Following removal, 5 cm x 5 cm nanofiberous PDLLA dressings coated with LFS were applied to the wound and held for 3 minutes under pressure. When the pressure was removed, the wound was hemostatic (B) despite the presence of large vasculature as visualized in the removed portion of the liver (C). After the surgery, the bandage was removed and the level of adherence evaluated. The adherence was strong enough to lift the liver by the bandage (D). Clot is clearly visible on the surface of the dressing (E).
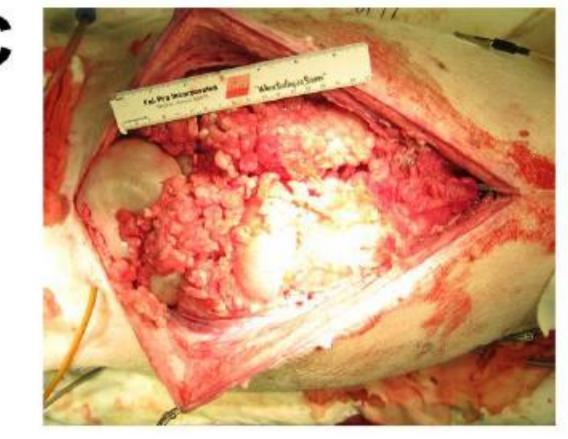

Figure 6. Delivery of fibrin sealant coated carrier foam to a swine abdominal cavity. Injection of the foam 30 seconds after liver resection. B) Swine abdominal cavity one hour after administration of first prototype carrier foam. C) Swine abdominal cavity one hour after administration of second prototype carrier foam.

1. Calcaterra, Jennifer, et al. "Recombinant human fibrinogen that produces thick fibrin fibers with increased wound adhesion and clot density." *Biomacromolecules* 14.1 (2012): 169-178.
2. Carlson, Mark A., et al. "A totally recombinant human fibrin sealant." *Journal of Surgical Research* 187.1 (2014): 334-342.

Conclusions

Swine injury models has proved to be an ideal method for testing various fibrin sealants. By removing the spleen of the swine, a coagulopathic model nearly identical to severe trauma in humans is obtained. Various applications of LFS have proven to be efficacious in restoring hemostasis for treatment of hepatic resection in swine.

Development of a Porcine Model of Severe Noncompressible Truncal Hemorrhage

U. R. Yanala, J.M. Johanning, I.I. Pipinos, W.H.Velander, M.A. Carlson.

University of Nebraska Medical Center
VA Nebraska - Western Iowa Health Care System

Omaha, Nebraska, USA
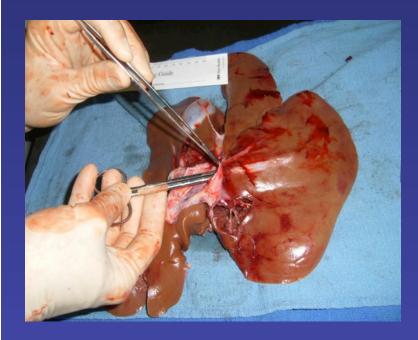
ASC 2014, San Diego.

- **AIM:** To develop a noncompressible truncal hemorrhage model which would produce exsanguination in ≤1 hour.

- **SUBJECTS:** Barrow swine, 3 months, 34-36 kg

- **TARGET ORGAN:** Liver, portal venous system.

Methods:

Injury 1: Central Stellate Injury

Injury 2: Excision of a portal vein branch distal to the main trunk

Injury 3: Near-transection of the left lateral lobe of the liver at its base.

Results:

Kaplan-Meier Survival Graph

Central Liver Injury (N=6)
Portal Vein Excision (N=6)
LLL Hemitransection (N=10)

Type of Injury	Final MAP, mm Hg (mean±sd)*	Blood loss, mL (mean ±sd)*	Final Hb, g/dL (mean±sd)*	Final INR (mean±sd)#
Central Liver Injury	65 ± 24	1140 ± 684	8.3 ± 2.7	1.1 ± 0.1
Portal Vein Excision	16 ± 4	3581 ± 353	1.1 ± 0.7	5.2 ± 4.4
Left Lateral Lobe Hemitransection	28 ± 16	2852 ± 657	3.3 ± 2.0	3.1 ± 3.7

MAP = mean arterial pressure; Hb = hemoglobin; INR = international normalized ratio; *$p < 0.05$, ANOVA; #$p < 0.05$, Kruskal-Wallis nonparametric analysis of variance.

Conclusions:

- Injury type 1 was not severe enough.

- Injury type 2 produced death too rapidly.

- Injury type 3 (left lateral lobe hemitransection) resulted in death by exsanguination in 40% of the subjects within 1 h, and evidence of severe hemorrhage in all subjects.

Questions ?

Submission to the 2015 CSA meeting (submitted 09/15/14)

EFFECT OF CRYSTALLOID INFUSION RATE IN A NONCOMPRESSIBLE HEMORRHAGE MODEL
Authors: Yanala, Johanning, Pipinos, Larsen, Velander, Carlson

Purpose
To determine the effect of the rate of fluid administration on survival, vital signs, blood loss, and laboratory parameters in a porcine model of noncompressible hemorrhage.

Methods
Twenty domestic swine (barrow, age 3 months, 32-36 kg) were anesthetized, had venous and arterial line placement, splenectomized through a midline incision, and then underwent hemitransection of the left lateral liver lobe (the noncompressible injury) without treatment. The incision was closed immediately after injury with towel clips. At 60 s after injury, warm Lactated Ringers solution was begun at either 150 or 20 mL/min IV (rapid and slow group, respectively, N = 10 per group); maximum volume was capped at 100 mL/kg. The rapid and slow group was monitored for 60 or 180 min, respectively, with continuous monitoring of vital signs and periodic lab draws.

Results
Pre-injury parameters (body weight, vital signs, hematology panel, coagulation panel, blood gas analysis, and splenic weight) did not differ between the two groups. Survival after one hour in both the rapid and slow groups was 60%; no further death occurred in the slow group with observation out to 180 min. Notable differences between the rapid and slow groups for select endpoints are shown in Table 1. Necropsy demonstrated that an equivalent number of portal vein and hepatic vein branches had been transected in each group. There were no significant differences between groups for heart rate, temperature, total volume of LR infused, or liver weight.

Conclusions
Although the two groups were not directly comparable (no formal randomization; longer observation time in the slow group), this study demonstrated that in a porcine model of noncompressible truncal hemorrhage, intravenous crystalloid resuscitation with a relatively slow infusion rate (20 mL/min) produced less blood loss and an improved laboratory profile (hemoglobin and protime), compared with resuscitation with a rapid infusion rate (150 mL/min). There was a nonsignificant trend of higher blood pressure in the slow group. While there was no effect on survival as reported, this endpoint might have been different if the rapid group had been followed for the same time period (180 min) as the slow group. This study supports the U.S. military's recent adoption of a "hypotensive resuscitation" protocol for warfighters injured in the field, which dictates that fluid resuscitation of an injured warfighter with hemorrhagic shock should be restricted until the subject arrives at a forward surgical unit. In other words, rapid/high-volume crystalloid resuscitation should be avoided in the early management of a subject with uncontrolled hemorrhage.

Table 1. Comparison of select endpoints between the rapid and slow groups.

Variable	Rapid Rate	Slow Rate	p-value
Survival	6/10	6/10	1.0000
Final MAP (mm Hg)	28 ± 17	44 ± 22	0.2571
Blood Loss (mL)	2,852 ± 657	1,548 ± 371	0.0002
Protime, 15 min (s)	16.9 ± 5.1	11.3 ± 1.4	0.0011
Final Hb (g/dL)	3.4 ± 2.0	6.3 ± 2.4	0.0155

STATE OF NEBRASKA

Dave Heineman
Governor

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February 4, 2014

Gustavo Larsen
LNK Chemsolutions, LLC
4701 Innovation Drive
Lincoln, NE 68521

RE: Nebraska Research and Development Initiative, Phase 2 Application
       <u>Notice of Approval – Grant No 14-01-110</u>

Dear Gustavo:

On behalf of Catherine D. Lang, Director of the Nebraska Department of Economic Development, it is a pleasure to inform you that LNK Chemsolutions, LLC has been approved for Nebraska R & D Phase 2 funding up to the amount of $400,000. The project activities include the testing and final development of hemostat products with an optimal configuration of friendly biological additives permitting regulatory approval by the U.S. Food and Drug Administration.

Before you proceed to request reimbursements under the terms of the Phase Two Research and Development Grant, it is important that you understand that all the Phase One requirements must be satisfied including reimbursement of any final expenditures made as a result of the activities described in the Phase One Grant. While Phase #2 costs may be incurred at this time, not all expenses may be reimbursable under the guidelines of the Nebraska R & D Initiative. Our Department will send you a contract with terms, and conditions on uses of the funds, reporting requirements, and the draw down process.

We congratulate LNK Chemsolutions on successfully obtaining Nebraska R & D funds. We look forward to working with you in carrying out your project.

If you have any questions regarding this information, contact me at (402) 471-3763 or by email at <u>mailto:stew.jobes@nebraska.gov</u>.

Sincerely,

Stew Jobes
Economic Development Program Manager
Nebraska Department of Economic Development

Copies: Luis Nunez, Joe Fox, DED

NEBRASKA DEPARTMENT OF ECONOMIC DEVELOPMENT
Catherine D. Lang, Director
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